Université de Montréal

Development and Psychometric Validation of Pain Scales in Feline Osteoarthritis

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Résumé

L'arthrose féline est détectable sur radiographie, surtout chez l'animal âgé. La rareté antérieure du diagnostic clinique s'explique par ses signes subtils et facilement attribués à d'autres maladies gériatriques ou au processus normal de vieillissement. Ces signes répondent néanmoins au traitement analgésique. Le but de ce projet de recherche était de développer et valider deux grilles de douleur arthrosique (*M*ontreal *I*nstruments for *C*at *A*rthritis *T*esting), une pour les propriétaires de chats [MI-CAT(C)], et une pour les vétérinaires [MI-CAT(V)]. Le développement était fondé sur une revue de la littérature, notre expertise clinique en douleur et en comportement félin, et un sondage de propriétaires de chats arthrosiques. Des experts internes et externes ont confirmé la validité de contenu des grilles. Ensuite, une étude pilote sur chats de laboratoire a permis une évaluation préliminaire de leur fiabilité et validité.

Dans le cadre d'un essai clinique chez des chats arthrosiques, la grille pour propriétaires MI-CAT(C) discriminait les groupes placebo et meloxicam, et ses changements de score corrélaient avec l'activité motrice et l'âge, soutenant sa validité. La grille était généralement facile à comprendre, appuyant de façon préliminaire sa validité de face (l'acceptabilité) et son interprétation. La mesure de fiabilité intra- et inter-observateur préconisait l'évaluation par le propriétaire principal *vs.* un(e) propriétaire secondaire. La grille MI-CAT(C) était homogène, sans redondance, selon l'évaluation préliminaire de la consistance interne.

Une seconde évaluation de la grille vétérinaire MI-CAT(V) a été menée chez des chats de laboratoire (avec ou sans arthrose naturelle). L'évaluation de la fiabilité intra- et inter-observateur démontrait une courbe d'apprentissage pour le nouvel utilisateur de la grille. Seules les sous-catégories *Gait* (démarche) et *Posture* (allure) avaient une tendance (non-significative) à détecter le statut arthrosique; la palpation et la manipulation des articulations n'avait aucune sensibilité du même genre. *Gait* et *Posture* corrélaient avec une mesure objective, la force verticale d'appui au sol.

Une analyse vidéo a ensuite été faite pour améliorer la sensibilité de la grille MI-CAT(V) à l'arthrose. La grille révisée a été soumise à des étapes successives de validation et

de raffinement, *via* trois études thérapeutiques (utilisant la gabapentine, le tramadol, et le meloxicam sous forme orale transmuqueuse par vaporisateur). Sa fiabilité intra- et inter-observateur, et l'évaluation préliminaire de la consistance interne étaient bonnes à excellentes, et elle fut capable de détecter le statut arthrosique. Cependant, elle ne détecta pas les effets thérapeutiques démontrés par d'autres mesures objectives.

Des recherches ultérieures devront confirmer que la grille pour propriétaires MI-CAT(C) distingue le statut arthrosique, et évaluer sa réponse, *vs*. placébo, à d'autres traitements que le meloxicam. La grille vétérinaire MI-CAT(V) requerra une confirmation de sa fiabilité et validité chez des chats de propriétaires ; elle nécessitera encore des raffinements pour détecter les effets de traitement. L'établissement de seuils (*p. ex. :* distinction arthrosique/non-arthrosique, différence minimale significative) pour les deux grilles est conseillé pour faciliter leur utilisation clinique, ainsi qu'une évaluation de leur faisabilité et utilité clinique, ainsi qu'une réévaluation de leur structure interne et de leur compréhension.

Mots-clés : psychométrie, métrologie, grille de douleur, chat, comportement, gériatrie, dégénérescence articulaire, douleur chronique, qualité de vie

Abstract

Radiographic signs of osteoarthritis are prevalent in cats, becoming more common with age. Historically, the rate of diagnosis has tended to be low, suggesting that signs are subtle and/or tend to be attributed to normal age-related changes or to other geriatric diseases. However, cats with osteoarthritis display signs that are responsive to analgesic treatment. This project aimed to develop and validate rating scales for detection and measurement of feline osteoarthritis pain and related disability (the *M*ontreal *I*nstruments for *C*at *A*rthritis *T*esting). Two such scales, one for use by caretakers/owners [MI-CAT(C)], and one for use by veterinarians [MI-CAT(V)], were developed based on a review of the literature, expert opinion, and a survey study of owners of cats with a diagnosis of osteoarthritis. The content validity (*via* expert review) was excellent for both scales. A pilot study in a colony of laboratory cats with naturally-occurring osteoarthritis evaluated reliability and ability to detect osteoarthritis status, for both scales; preliminary revisions were made based on the results.

The MI-CAT(C) owner scale subsequently underwent validation in a clinical trial of meloxicam in client-owned osteoarthritic cats. Evidence for validity included the ability to distinguish placebo from active treatment, and correlations with objectively measured activity and age. Owners found most scale items clear/easy to understand, preliminarily supporting comprehensibility and face validity (acceptability). Evaluation of intra- and inter-rater reliability suggested that secondary owners varied substantially in their ability to complete the scale, compared to primary owners. A preliminary assessment of internal consistency reliability supported homogeneity, without redundancy, of the scale.

The MI-CAT(V) veterinary scale was evaluated in a study of laboratory cats with and without naturally-occurring osteoarthritis. Intra- and inter-rater reliability assessments suggested that a naïve user's ability to use the scale was influenced by experience with it. The scale was unable to distinguish osteoarthritic and non-osteoarthritic cats, but the subcategories Gait and Posture were somewhat promising based on a non-significant tendency to detect osteoarthritis status, and correlations with an objective measure of osteoarthritis pain, peak vertical force. Palpation of the limbs did not detect osteoarthritis status.

A video analysis was performed to increase MI-CAT(V) scale sensitivity to osteoarthritis. Subsequent evaluation and refinements based on three therapeutic trials (involving gabapentin, tramadol, and oral transmucosal meloxicam treatments) in laboratory cats with and without naturally-occurring osteoarthritis resulted in good to excellent intra- and inter-rater reliability, and ability to detect osteoarthritis status. Preliminary evidence supported scale internal consistency. Therapeutic response detected by objective outcome measures was not demonstrable using the scale.

It is recommended that the MI-CAT(C) owner scale be evaluated for ability to distinguish osteoarthritic from non-osteoarthritic cats. The MI-CAT(V) veterinary scale requires testing in client-owned cats, and potentially further refinements to permit detection of treatment effects, if it is to be used as more than a disease screening tool. Both scales require additional investigation of internal structure and comprehensibility, and determination of cut-points to guide clinical use (*e.g.*, minimally important difference, and thresholds for classification of cats as osteoarthritic *vs.* non-osteoarthritic), and evaluation of their feasibility and clinical utility.

Keywords: psychometric, metrology, pain scale, cat, behavior, geriatric, degenerative joint disease, chronic pain, quality of life

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5-HT3	5-hydroxytryptamine (serotonin) 3 receptor
α	Alpha
AAFP	American Association of Feline Practitioners
AAHA	American Animal Hospital Association
ACG	Anterior cingulate gyrus
am	*ante meridiem*
AM	Telemetric locomotor activity monitoring
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ASIC	Acid-sensing ion channel(s)
ATP	Adenosine triphosphate
β	Beta
BCS	Body condition score
BIV	Boehringer Ingelheim Vetmedica, Inc
BML	Bone marrow lesions
BP	Arterial blood pressure
BPB	Body Posture-Back subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)
BPF	Body Posture-Forelimbs subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)
BPH	Body Posture-Hind Limbs subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)
BSAVA	British Small Animal Veterinary Association
CBC	Complete blood count
CCLT	Cranial cruciate ligament transection
CGRP	Calcitonin gene-related peptide
CI	Confidence interval
CKD	Chronic kidney disease
CMPS-F	Composite Measures Pain Scale – Feline (University of Glasgow)

CNS	Central nervous system
COX	Cyclo-oxygenase
CSOM	Client-Specific Outcome Measures scale
δ	Delta
D	Day
DAM	Daytime locomotor activity monitoring
DF	Degrees of freedom
DIVAS	Dynamic and interactive visual analogue scale
DJD	Degenerative joint disease
DLSS	Degenerative lumbosacral stenosis
DMOAD	Disease-modifying osteoarthritis drug
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglion
DYAR	Dog-years at-risk
e.g.	*exempli gratia*
EEG	electroencephalography
et al.	*et alia*
FACS	Facial Action Coding System
FDA	Food and Drug Administration (United States of America)
FI	Intact female
FMPI	Feline Musculoskeletal Pain Index
fMRI	Functional magnetic resonance imaging
FS	Spayed female
GABA	Gamma-aminobutyric acid
GDE	Global Distance Evaluation (lameness) subcategory (Surgeon's orthopedic evaluation, Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)
GF	Gait-Forelimbs subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)

GG	Gait-General subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)
GH	Gait-Hind Limbs subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)
GLM	Green-lipped mussel
GLM	Generalized linear model
h	Hours
H_0	Null hypothesis
H_1	Alternate hypothesis
HR	Heart rate
i.e.	*id est*
IACUC	Institutional Animal Care and Use Committee
IASP	International Association for the Study of Pain
ICC	Intra-class correlation coefficient
IL	Interleukin
IVAS	Interactive visual analogue scale
IVD	Intervertebral disc
KR-20	Kuder-Richardson formula 20
M	Meloxicam
MCID	Minimum clinically important difference
MDC	Minimally detectable change
MGS	Mouse Grimace Scale
MI	Intact male
MI-CAT(C)	Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner
MI-CAT(C)-v1	Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner, version 1
MI-CAT(C)-v2	Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner, version 2
MI-CAT(V)	Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians

MI-CAT(V)-v1	Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians, version 1
MI-CAT(V)-v2	Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians, version 2
MI-CAT(V)-v3	Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians, version 3
MI-CAT(V)-v4	Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians, version 4
MI-CAT(V)-v5	Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians, version 5
MIC	Minimally important change
MID	Minimally important difference
min	Minutes
MN	Neutered male
MR	Magnetic resonance
MRI	Magnetic resonance imaging
NAM	Night-time locomotor activity monitoring
NE	Not evaluable
NGF	Nerve growth factor
NK-1	Neurokinin-1
NMDA	*N*-methyl-D-aspartate
NO	Nitric oxide
NRS	Numerical rating scale
NS	Not significant
NSAID	Nonsteroidal anti-inflammatory drug
NSERC	National Sciences and Engineering Research Council of Canada
O	Osteophyte
OA	Osteoarthritis, osteoarthritic
OB	Other Behaviors subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)

OTMS	Oral transmucosal meloxicam spray
P	Placebo
PAG	Periaqueductal grey
Pentosan PS	Pentosan polysulfate
PET	Positron emission tomography
PG	Prostaglandin
PGE_2	Prostaglandin E2
pm	*post meridiem*
PPAR	Peroxisome proliferator-activated receptors
Pr	Probability
PSGAG	Polysulfated glycosaminoglycans
PVF	Peak vertical force
PWT	Von Frey-anesthesiometer-induced paw withdrawal threshold
QOL	Quality of life
QST	Quantitative sensory testing
Rho_S	Spearman's Rho
RMTS	Response to mechanical temporal summation
ROI	Region of interest
RVM	Rostroventral medulla
s	Seconds
SC	Serum chemistry
SD	Standard deviation
SDS	Simple descriptive scale
SE	Standard error
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
SOE	Surgeon's orthopedic evaluation
SSC	Secondary somatosensory cortex

SUHF	Standing up on Hind Feet to Investigate a Higher Surface subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)
T1w-GRE	T1-weighted three-dimensional fast gradient recalled echo
T4	Thyroxine
THAL	Thalamus
TNF-α	Tumour necrosis factor-α
TRP	Transient receptor potential
TRPA1	Transient receptor potential cation channel, subfamily A (ankyrin), member 1
TRPM8	Transient receptor potential, subfamily M (melastatin), member 8
TRPV	Transient receptor potential cation channel, subfamily V (vanilloid)
TRPV1	Transient receptor potential cation channel, subfamily V (vanilloid), member 1
UNESP-Botucatu MCPS	Universidad Estadual Paulista (São Paulo State University)-Botucatu Multidimensional Composite Pain Scale
VAS	Visual analogue scale
VCA	Veterinary Centers of America
VF	Von Frey punctate withdrawal threshold
VIP	Vasoactive intestinal polypeptide
VRS	Verbal rating scale
vs.	*versus*
VTH	Veterinary Teaching Hospital
W	Week
WDR	Wide dynamic range
WEHM	Willingness and Ease of Horizontal Movements subcategory (Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians)
wKappa	Weighted Kappa

Dedication

For Meike (my best girlie), Baker (sweetest, baddest kitty), and Drew. Thanks for the love, the shared joys, and the inspiration.

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To the cats.

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1. INTRODUCTION

This project was conceived at a time of increasing interest in the problem of feline osteoarthritis (OA), in the veterinary community. In the preceding decade, numerous reports had been published that suggested that cats had a high prevalence of radiographic signs of OA, despite a generally low rate of diagnosis. As OA was commonly diagnosed in humans and other animals, the implication appeared to be that signs were particularly difficult to recognize in this species. This research project therefore undertook to develop and validate pain scales to facilitate detection and measurement of the severity of feline clinical OA.

This introductory section consists of five parts. The first is a review article that describes the biology of pain, "*The physiology and pathophysiology of pain*" (Klinck MP, Troncy E). It was published in the *British Small Animal Veterinary Association (BSAVA) Manual of Canine and Feline Anaesthesia and Analgesia* (Editors: Duke-Novakovski T, Vries M, Seymour C. 3rd ed. BSAVA: Wiley; 2015. Pages 97-112). Author contributions were as follows: Mary Klinck contributed to the conception of the article, wrote the initial article manuscript, and participated in revising it; Eric Troncy was invited to contribute a manuscript, and participated in the conception, writing of, and revisions to the article manuscript.

The second part provides an overview of pain rating scales. This includes descriptions of common types, as well as an explanation of the methods of development and the theory and processes involved in scale validation. The third part reviews the current understanding of feline OA, and covers its epidemiology, the pathology and structural changes associated with the disease, and reported clinical signs and objective and subjective measures for its assessment.

The fourth part of the introductory section consists of a review article, "*Translational pain assessment: Could natural animal models be the missing link?*" (Klinck MP, Mogil JS, Moreau M, Lascelles BDX, Flecknell P, Poitte T, Troncy E), published in *PAIN* (2017, 158(9): 1633-1646; doi: 10.1097/j.pain.0000000000000978), and selected as an Editor's Choice article. It discusses the translational potential of naturally-occurring painful disease in

veterinary patients, such as OA in cats. It expands on the topics of pain assessment in animals, and discusses inter-species similarities in OA and other painful diseases. Author contributions were as follows: Mary Klinck contributed to the conception of the review article, wrote the initial article manuscript, and coordinated revisions to it. Jeffrey Mogil, Duncan Lascelles and Paul Flecknell all contributed to the conception of the review article, and participated in the writing and revising of the article manuscript. Maxim Moreau and Thierry Poitte participated in the writing and revising of the article manuscript. Eric Troncy initiated the work, contributed to the conception of the review article, participated in writing the initial article manuscript and in revising it, and oversaw the work as a whole.

The fifth and final part of this introduction describes the hypothesis and research objectives of this project.

Following the introduction, four primary research articles will be presented that describe the work conducted to develop and validate two feline OA pain scales. Finally, the results will be discussed, along with limitations of the project and recommendations for future research, with emphasis on ways to refine and further validate the presented feline OA pain scales.

All articles included in this Thesis are presented as published/submitted for publication (*e.g.*, with respect to the version of English spelling and wording used), and have not been modified for inclusion in this work.

1.1 The Physiology and Pathophysiology of Pain

Klinck MP[1] and Troncy E[1]

1.1.1 Introduction

Pain in humans is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience, associated with actual or potential tissue damage, or described in terms of such damage" (IASP Editorial 1979). This definition reflects the multidimensional nature of pain and that it is not just a sensory experience. Because the IASP definition relies heavily on the individual describing the pain (*i.e.* self-reporting), an alternative definition of pain is needed for animals. Molony and Kent (1997) proposed the following definition: "animal pain is an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence, and to promote recovery" (Molony and Kent 1997).

Pain management in animals has improved over the past 2–3 decades. Previously, there was a tendency both to under-recognize and to under-treat animal pain (Flecknell 2008). Vertebrate animals share a common anatomy and physiology involved in pain processing; therefore, injuries, diseases and procedures that are painful in humans are likely to be painful in animals. In addition, while physiological adaptive pain can serve a protective function, uncontrolled pain can impede healing and lead to long-term complications.

[1] GREPAQ – Department of veterinary biomedical sciences, Université de Montréal, P.O. box 5000, St.-Hyacinthe (Quebec), Canada J2S 7C6

A variety of pain types are encountered in cats and dogs, including:

- Short-lasting procedural pain (*e.g.* that due to venepuncture)
- Acute pain associated with injury, illness or surgery
- Chronic pain associated with chronic disease states *e.g*. osteoarthritis (OA).

Pain is normally proportional to the degree of stimulus, injury or other disease state, but pain and its intensity may also:

- Exceed the stimulus
- Outlast healing or treatment of the inciting cause
- Be present even in the absence of a stimulus.

Such altered pain states tend to be associated with changes in nociceptive processing within the central nervous system (CNS). These changes often play a role in many chronic painful diseases and may explain differences between individuals in the experience of pain intensity produced by similar, detectable pathology (Phillips and Clauw 2011). In humans, chronic pain may affect the ability to perform certain tasks, and cause sleep disturbance and affective problems such as depression and anxiety (Hadjistavropoulos and Craig 2002). Comparable sequelae may be present in animals, but are less easily recognized. Understanding the mechanisms of pain helps the practitioner to plan appropriate analgesic protocols and to interpret signs of pain in animals.

1.1.2 Definitions for types of pain

1.1.2.1 Evolution of definitions

Pain has diverse aetiologies and there is no unifying theory for its various manifestations. Previous definitions of pain were too restrictive, and pain is best described as a combination of various pain types. Most types of pain are in fact of ‘mixed origin’ and often involve a combination of neuropathic pain with nociceptive and/or inflammatory components; for example, neoplasia can cause mixed pain through a combination of inflammation and the local destruction of tissues and nerves. Many chronic pain states, including those that were

previously thought to have purely inflammatory aetiologies (*e.g.* OA) actually involve mixed pain. It also appears that physiological, protective, nociceptive pain can lead to pathological, deleterious, chronic pain if it is not adequately recognized and treated. Persistent postsurgical pain has characteristics of neuropathic pain (Marchand 2008, Woolf 2004), but the contribution of the neuropathic component varies with the type of surgery and probably depends on the degree of surgical nerve injury (Haroutiunian, Nikolajsen et al. 2013).

1.1.2.2 Nociceptive (acute) pain

This type of pain is also referred to as physiological, normal, adaptive or protective pain. It occurs when a potentially injurious (noxious) stimulus is applied to the body (Woolf 2011), and has an intensity and duration proportional to the stimulus (Latremoliere and Woolf 2009). It usually produces a protective response (*e.g.* limb withdrawal, behavioural avoidance strategies) and, if no actual injury occurs, stops when the external stimulus is removed. The descriptor 'acute' refers to a pain sensation that is temporary.

An example of nociceptive pain is the pain produced by pinching the skin.

1.1.2.3 First pain

This is the immediate pain that occurs following the activation of thinly myelinated (rapidly conducting) Aδ nociceptor fibres. It is commonly described by people as sharp, pricking or stabbing in nature (Meintjes 2012).

1.1.2.4 Second pain

This is pain associated with the activation of unmyelinated (slowly conducting) C nociceptor fibres, and is therefore perceived after first pain. It is commonly described as slow or burning in nature.

1.1.2.5 Chronic pain

This term can refer to pain that either outlasts the original tissue injury and the expected healing time or lasts longer than a specified period, generally 3 months. Because of the large differences in life expectancy of different animal species, the first definition may be more applicable for describing chronic pain in animals. Chronic pain is often associated with changes in central pain processing (Phillips and Clauw 2013).

1.1.2.6 Inflammatory pain

This type of pain is associated with tissue injury or immune cell activation. Chemical changes in the tissues around the nociceptors either facilitate, or directly cause, nociceptor activation. Pain from a surgical wound and the surrounding tissues is an example of inflammatory pain.

1.1.2.7 Functional (idiopathic) pain

This is pain that arises in the absence of a detectable tissue or nerve injury; it is therefore difficult to recognize and is rarely diagnosed in animals (Price and Nolan 2007). It is also called maladaptive or psychogenic pain. Fibromyalgia in humans is associated with functional pain.

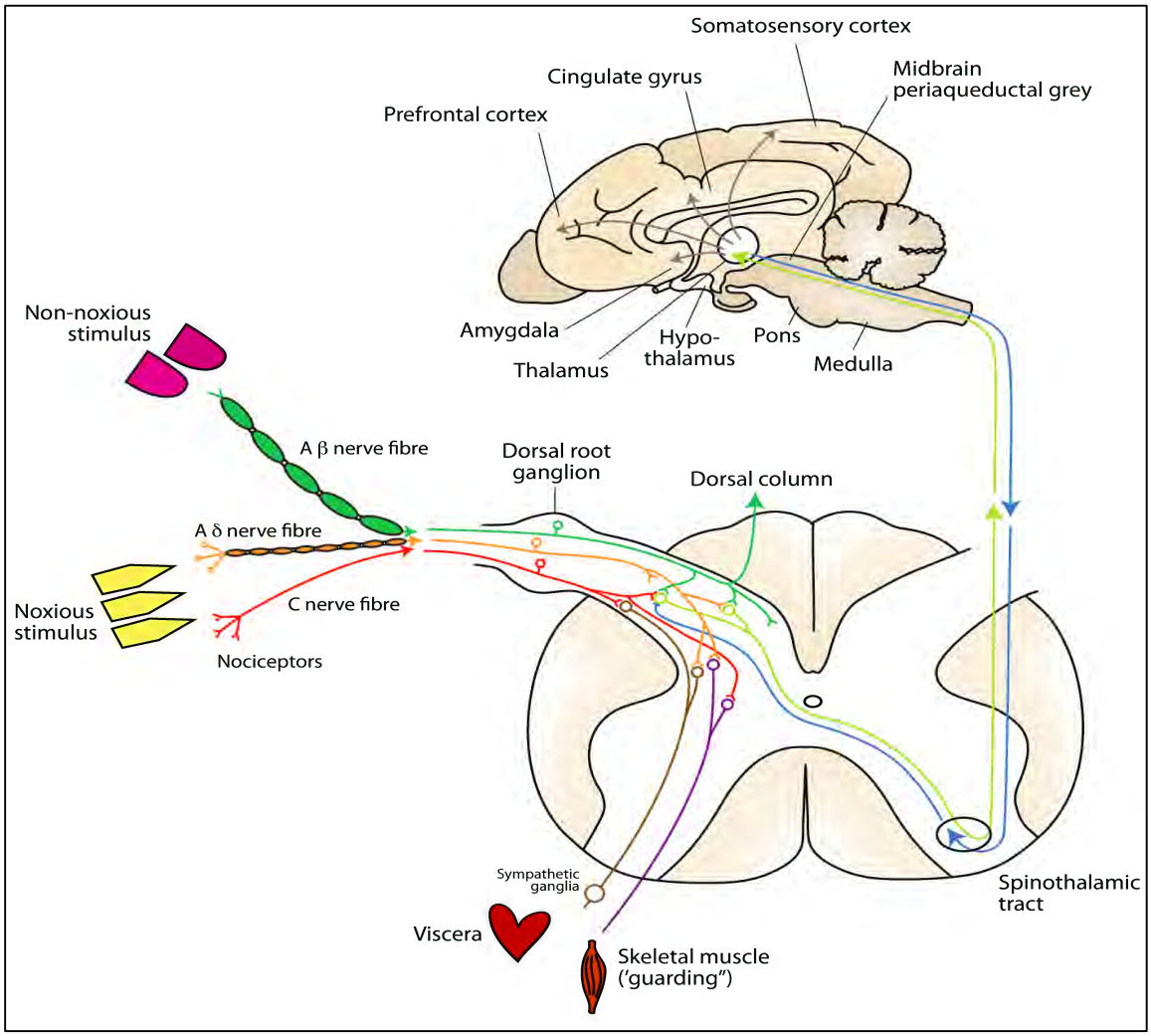
1.1.2.8 Neuropathic pain

This type of pain is initiated or caused by a primary lesion or dysfunction within the nervous system. Secondary changes in both affected and non-affected neurons result in the facilitation or direct activation of nociceptors. Examples of conditions that may be associated with neuropathic pain are diabetic neuropathy and nerve transection (*e.g.* post-amputation pain).

1.1.3 Processing nociceptive information

The nociceptive pathway consists of peripheral components – nociceptors and their first-order neuron – and complex central components, which consist of second-order and third-order neurons, ascending and descending pain pathways, and internuncial neurons connecting with peripheral nerves to other systems such as viscera and skeletal muscle (Figure 1.1.1).

Figure 1.1.1: Diagram illustrating the ascending and descending nociceptive pathways with connections within the spinal cord to the autonomic nervous system and skeletal muscle.



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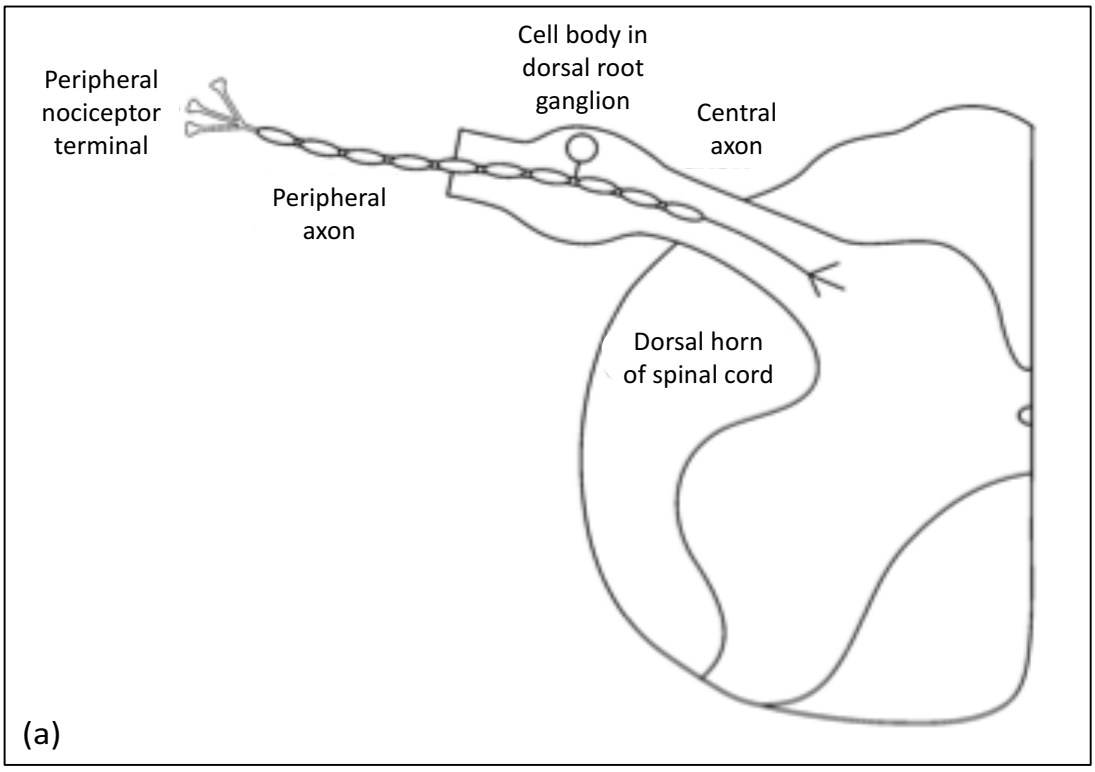
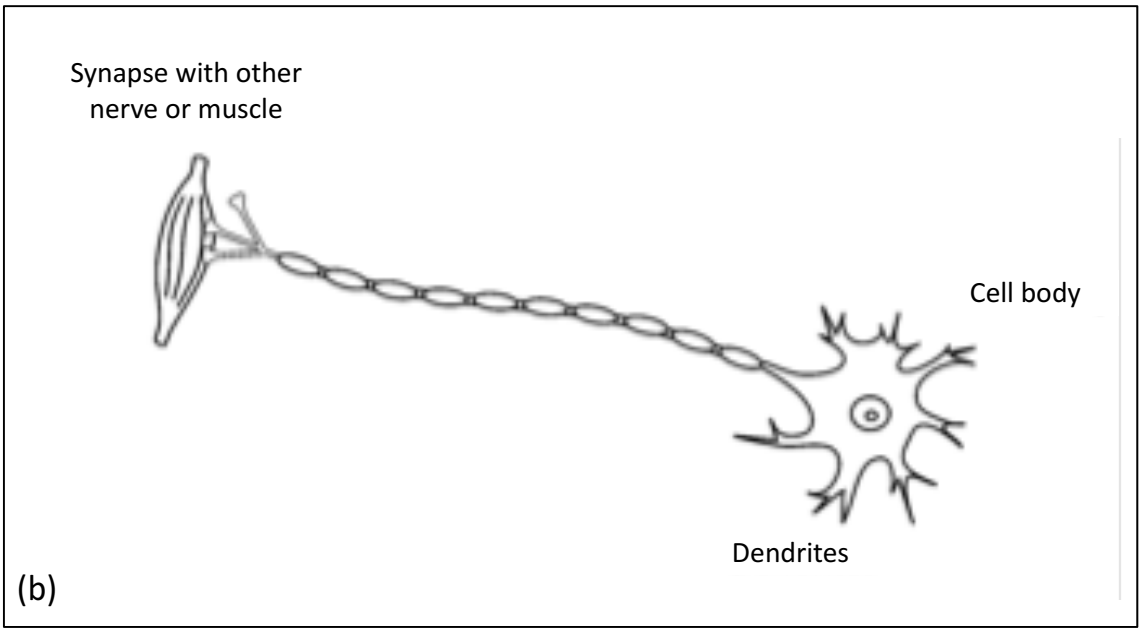
1.1.3.1 The nociceptor and first-order neuron

Nociceptor neurons consist of:

- Specialized, branching, unencapsulated axon terminals in the target tissue (nociceptor)
- An axon (fibre)
- A cell body in the dorsal root ganglion (DRG)
- A central terminal in the dorsal horn of the spinal cord (Figure 1.1.2a) (Gold and Gebhart 2010).

Figure 1.1.2: Diagram of (a) a nociceptive first order neuron and (b) a prototypical neuron, illustrating structural differences.





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They differ from the prototypical neuron, which has a receiving end (dendrite) and a transmitting end (axon) (Figure 1.1.2b). Instead, nociceptor neurons have a pseudo-unipolar structure, with the cell body connected *via* a common axonal stalk to both the peripheral and the central terminals; this permits bidirectional transmission of information (Basbaum, Bautista et al. 2009). The term *dromic* refers to impulse transmission in the normal direction, and *antidromic* is transmission in the opposite direction.

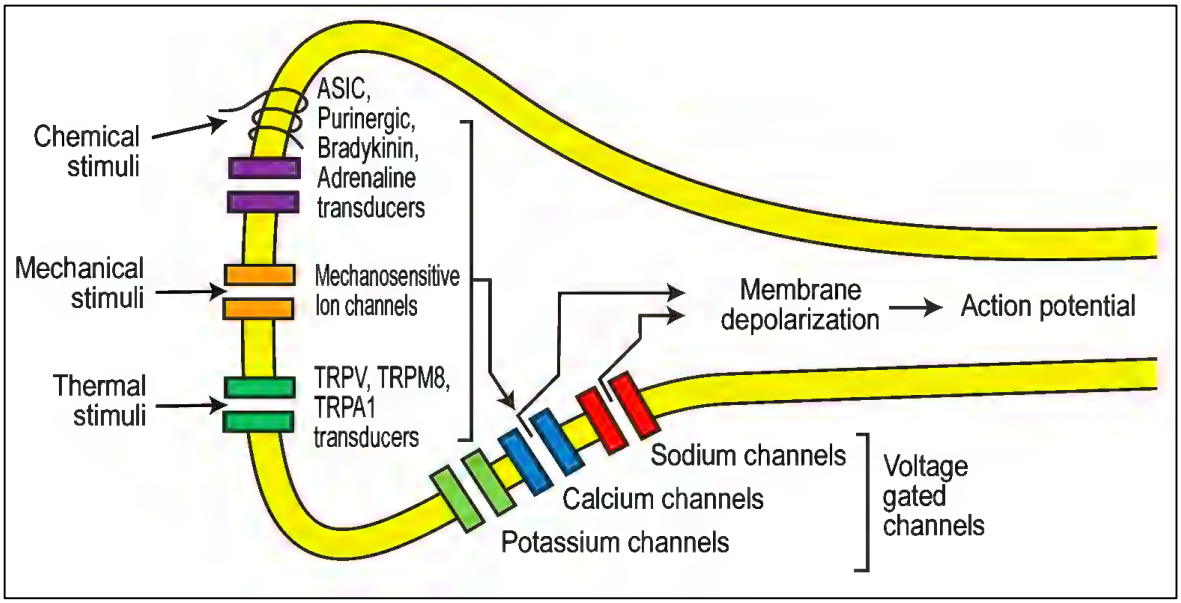
1.1.3.1.i Nociceptor

Nociceptors are present in the skin, muscles, joints and viscera, with the highest numbers being in the skin. They are not found in the brain, except in the meninges (Bear, Connors et al. 2007). Their responsiveness varies according to the site and tissue type (Julius and Basbaum 2001), as well as with the type and strength of the stimulus. For example, cutting and crushing injuries activate nociceptors in the skin, but not necessarily those in the joints, muscle and viscera, while rotation and distension more reliably activate joint and visceral nociceptors. It is important to appreciate that although nociceptors are often called

pain-sensing neurons, they merely indicate the presence of potentially harmful stimuli; it is the brain that interprets the signal as painful.

Normal nociceptive processing begins when a potentially injurious stimulus (*e.g.* mechanical, thermal, electrical or chemical) activates nociceptor cell membrane molecular structures (transducers) (Figure 1.1.3). Once triggered, the transducers cause membrane ion channels to open, allowing sodium and calcium ions to move down their respective concentration gradients and resulting in membrane depolarization. If the noxious stimulus is of sufficient amplitude and duration to produce an action potential, an impulse will travel towards the CNS to the dorsal horn of the spinal cord. Flow of potassium ions through membrane channels acts to resist spontaneous depolarization in the resting state; therefore, blockade of potassium channels may also contribute to action potential formation.

Figure 1.1.3: Diagram of a nociceptor terminal illustrating various transducers sensitive to noxious stimuli and ion channels.



Legend: Influx of calcium and sodium ions in sufficient concentration will cause an action potential along the nerve axon. Potassium ions are usually inhibitory. ASIC = acid-sensing ion channels; TRP = transient receptor potential. (© Juliane Deubner, University of Saskatchewan, Canada)

Most nociceptors are polymodal, that is, they respond to multiple types of stimuli. There are also 'silent' nociceptors that become responsive only after they have been sensitized by tissue injury; these have been linked to 'mechanically insensitive afferents' of Type II Aδ (~50%) and C (~30%) fibres (see below) (Dubin and Patapoutian 2010).

Various channels are associated with stimulus transduction at the nociceptor terminals (Figure 1.1.3). These include: mechanosensitive cation channels; purinergic channels (sensitive to adenosine triphosphate (ATP)); acid-sensing ion channels (ASIC); and various transient receptor potential (TRP) ion channels that can detect noxious heat (TRP vanilloid (TRPV) channels, particularly TRPV1) and pressure (TRPV channels), noxious cold (TRP melastatin-8 (TRPM8) and TRPA1 channels), and various chemicals (TRPV1, TRPA1 and TRPM8 channels). Other substances in the tissues (*e.g.* inflammatory mediators), as well as influences from other channels, can modulate the sensitivity of transduction channels.

Transduction channels can therefore be opened directly (*e.g.* by protons or capsaicin) or indirectly (*via* G-protein-coupled receptors and tyrosine kinase receptors). Receptor potentials generated by transduction of noxious stimuli activate voltage-gated ion channels, leading to the generation of an action potential.

1.1.3.1.ii First-order neuron

Nociceptive signals are normally transmitted from the periphery to the spinal cord by two types of nociceptive axon fibres: Aδ fibres (thinly myelinated, 1–5 μm diameter) and C fibres (unmyelinated, 0.2–1.5 μm diameter). The Aδ fibres have relatively rapid transmission speeds (~20 m/s) and are responsible for conducting first pain (which should not be confused with 'first'-order neurons). The C fibres have slow transmission speeds (<2 m/s) and are responsible for conducting second pain. The Aδ nociceptors may be classified as:

- Type I: Respond to chemical and mechanical stimuli, but have a higher heat threshold compared with Type II unless they are sensitized by tissue injury
- Type II: Have a lower heat threshold compared with Type I, but a higher mechanical threshold.

Most C fibres respond to noxious chemical stimuli, such as protons, and to thermal and mechanical stimuli, but some are mechanically insensitive unless they have been sensitized by tissue injury. Peptidergic C fibres release substance P and calcitonin gene-related peptide (CGRP), and have receptors for neural cell derived-nerve growth factor (NGF). Non-peptidergic C fibres carry receptors for glial-derived neurotrophic factor. Peptidergic neurons are thought to be involved in inflammatory pain and antidromic 'neurogenic inflammation' (see below) (Chiu, von Hehn et al. 2012). The non-peptidergic neurons may have greater involvement in neuropathic pain (Golden, Hoshi et al. 2010). There is no distinction between Aδ and C fibres in visceral pain, which means that no first and second pain occurs and the pain tends to be poorly localized.

Other sensory neurons (pressure, proprioception) transmit information *via* large-diameter, myelinated, rapidly conducting Aβ fibres. In the normal state, these Aβ sensory neurons do not transmit pain signals, and their stimulation may even reduce nociceptive transmission: for example, rubbing a painful area can actually reduce pain.

1.1.3.1.iii Pharmacological application

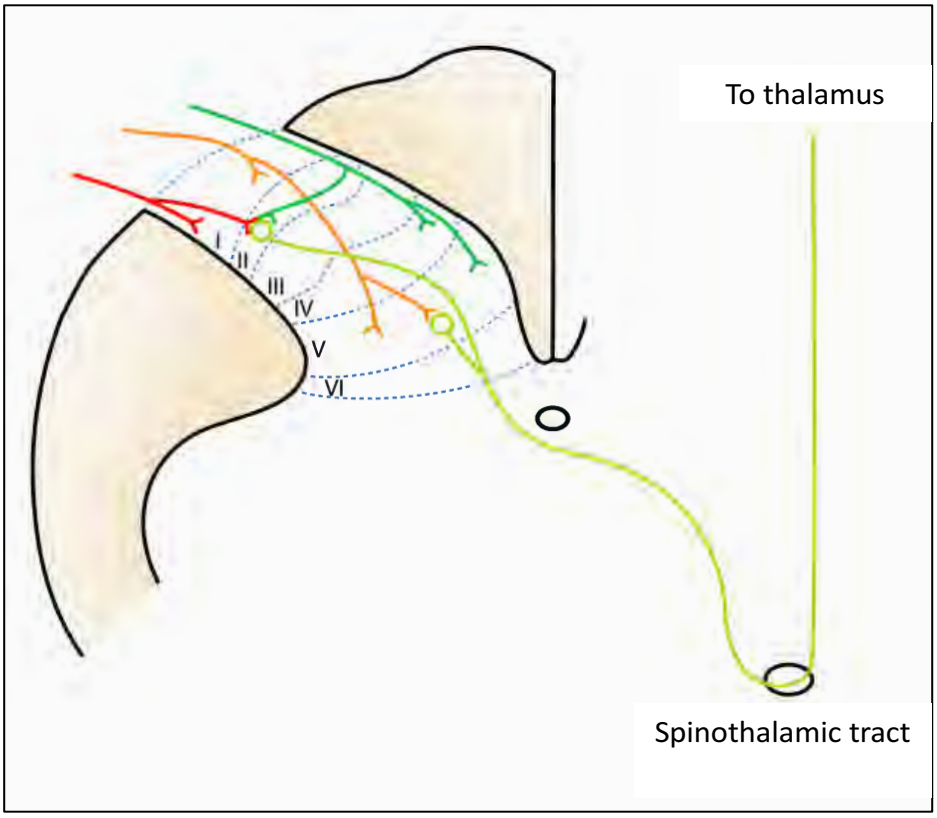
Capsaicin (the active component of chilli peppers) can open the TRPV1 channel, but with repeated or prolonged application it causes persistent functional desensitization of the polymodal primary nociceptors associated with TRPV1 activation. Clinically, this mechanism enables the topical application of capsaicin or eugenol (extracted from clove oil), and intrathecal administration of resiniferatoxin (extracted from resin spurge, a cactus-like plant commonly found in Morocco), to be effective.

Capsaicin can therefore be used as a base in which to formulate analgesic molecules. For example, by binding to TRPV1, capsaicin allows the normally ineffective positively charged molecule QX314 (an analogue of lidocaine) to enter neuronal cells and block voltage-gated sodium channels. This mechanism might enable the development of agents that selectively block voltage-gated sodium channels (local anaesthetics, as well as anticonvulsants such as phenytoin or carbamazepine) or N-type calcium channels such as gabapentinoids (gabapentin and pregabalin).

1.1.3.2 The spinal cord and brain

The spinal cord consists of a central canal filled with cerebrospinal fluid surrounded by the grey matter (divided into the dorsal, lateral and ventral horns) and the more peripheral white matter (Figure 1.1.4). The dorsal horn is composed of sensory nuclei that receive and process incoming somatosensory information. It is anatomically divided into 10 laminae, based on layer identifications made by the neuroscientist Bror Rexed.

Figure 1.1.4: Diagram of a transverse section of the spinal cord, illustrating the central terminals of the first order Aß (green), Aδ (orange) and C (red) neurons within the dorsal horn of the grey matter.



Legend: The Roman numerals represent the position of the terminals in Rexed's laminae of the spinal cord. (© Juliane Deubner, University of Saskatchewan, Canada)

1.1.3.2.i Dorsal horn neurons

Nociceptive fibres arriving from the periphery have their cell bodies located either in the DRG of the spinal cord (for neurons innervating most of the body) or in the trigeminal ganglia (for those innervating the head). Their central axonal projections extend into the spinal grey matter to communicate either with second-order neurons located in the dorsal horn, or with the trigeminal subnucleus caudalis in the caudal medulla. Descending pathways have been identified that play a role in pain transmission modulation, mainly through an inhibitory action. Dorsal horn neuron pain signal output therefore depends on the complicated interplay of excitatory inputs and inhibition by spinal interneurons (Kuner 2010).

Second-order neurons consist of interneurons, neurons of ascending tracts to the brain, intersegmental neurons and projecting neurons, and α-motor neurons involved in reflex withdrawal responses. These connections are partly responsible for muscle guarding of injured sites, withdrawal reflexes and changes within the autonomic nervous system. The Aδ fibres synapse with second-order neurons in Rexed's laminae I and V of the dorsal horn, C fibres synapse in the superficial laminae I and II, and non-nociceptive Aβ fibres synapse in laminae II, IV, and V.

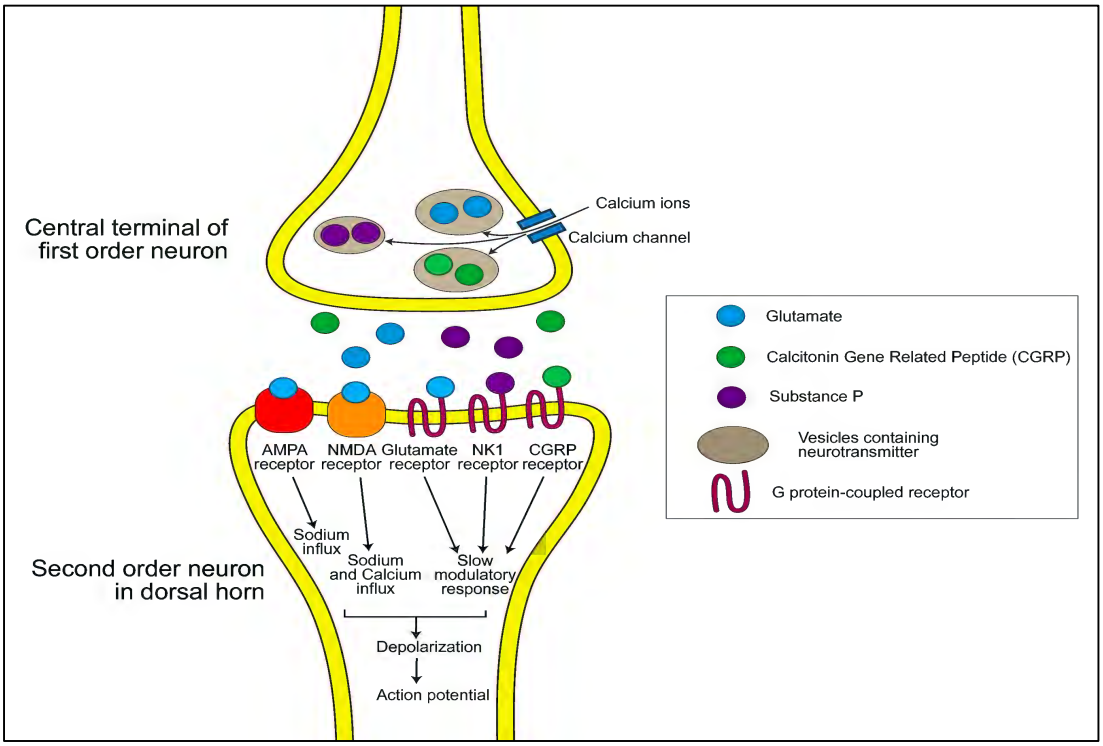
Lamina V contains wide dynamic range (WDR) neurons that respond to both noxious and non-noxious stimulation. The WDR neurons are activated by weak stimuli, but respond with increasing discharge frequency as the intensity of the mechanical stimulus increases. The WDR neurons are important in the descending control of pain, and their sensitization by repetitive nociceptive stimulation plays a key role in the induction of long-term inflammatory and/or neuropathic pain states (Millan 2002). The WDR neurons also receive visceral input, which explains why visceral pain can be referred to somatic sites, such as the pain in the left arm associated with angina in humans.

1.1.3.2.ii Dorsal horn synaptic transmission

Direct excitatory and/or neuromodulatory neurotransmitters are released at synapses in the dorsal horn in quantities proportional to the degree of nociceptor stimulation, to activate

receptors on the second-order neuron (Figure 1.1.5). Glutamate is the main excitatory neurotransmitter (Muir and Woolf 2001). It acts on the kainate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) classes of ionotropic (also known as ligand-gated ion channels) glutamate receptor, and also on metabotropic (G-protein-coupled) glutamate receptors. Other neurotransmitters are also involved, such as substance P, which binds to the neurokinin-1 (NK-1) receptor.

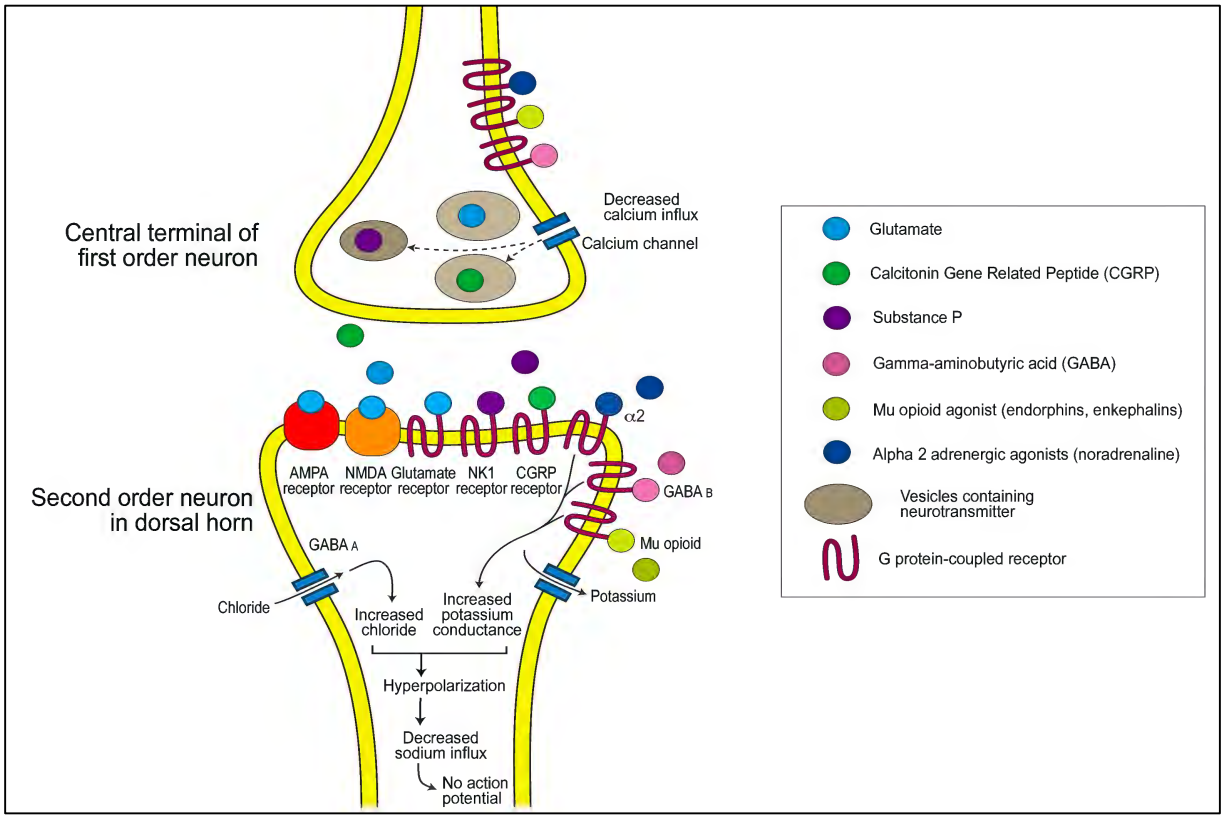
Figure 1.1.5: Diagram of a synapse between first- and second-order nociceptive neurons.



Legend: Receptors for AMPA and NMDA are ionotropic for glutamate (as is the kainite receptor, not shown on the diagram). The glutamate receptor is metabotropic for glutamate. The metabotropic receptors NK-1 and CGRP are for substance P and calcitonin gene-related peptide (CGRP), respectively. AMPA = α-amino-hydroxy-5-methyl-4-isoxazolepropionic acid; NK-1 = neurokinin-1; NMDA = *N*-methyl-D-aspartate. (© Juliane Deubner, University of Saskatchewan, Canada)

Inhibitory substances (Figure 1.1.6) include gamma-aminobutyric acid (GABA), which decreases neuronal excitability both pre- and postsynaptically through the activation of $GABA_B$ and $GABA_{A,B}$ receptors, respectively. Enkephalin acts in an inhibitory manner on presynaptic voltage-gated calcium channels in the primary afferent nerve terminal. Glycine is primarily inhibitory, but is also required as a co-agonist with glutamate to activate NMDA receptors.

Figure 1.1.6: Diagram showing details of the inhibitory effects between the terminals of first- and second-order neurons within the dorsal horn of the spinal cord.



Legend: Alpha-$_2$ adrenergic, GABA and mu opioid receptor stimulation decreases chance of an action potential developing in second-order neuron. AMPA = α-amino-hydroxy-5-methyl-4-isoxazolepropionic acid; NK-1 = neurokinin-1; NMDA = *N*-methyl-$_D$-aspartate. (© Juliane Deubner, University of Saskatchewan, Canada)

The three main opioid receptors (mu, delta, kappa) and alpha-2 adrenergic receptors are co-localized on both pre- and postsynaptic terminals (Riedl, Schnell et al. 2009). Presynaptic opioid receptor activation is associated with decreased calcium influx and decreased release of neurotransmitter into the synapse (Figure 1.1.6). Postsynaptic opioid receptor activation is associated with hyperpolarization (as a result of opening potassium ion channels), which leads to decreased action potential generation and inhibits second-order neuronal activation.

1.1.3.2.iii Pharmacological application

Spinal neuronal transmission is the target of many therapeutic interventions, which aim to reduce nociceptive transmission and sensitization processes. Local anaesthetics mainly target the voltage-gated sodium channels. Blocking the sodium channels with anticonvulsants or certain antidepressants inhibits nociceptive conduction and can be useful in the treatment of neuropathic pain. Anticonvulsants can also bind to spinal $GABA_A$ receptors and to cannabinoid receptors to reduce the synaptic release of glutamate.

The anatomical co-localization of opioid and alpha-2 adrenergic receptors at both spinal and supraspinal levels, and the sharing of similar signalling pathways with similar cellular actions, may underlie the mechanism of pharmacological synergism observed with analgesic agents that act at these two receptor types (Chabot-Dore, Schuster et al. 2015).

Ziconotide, an agent derived from the venom of a Pacific Ocean cone snail, inhibits the N-type calcium channel, which is present throughout the nervous system. When used in humans, to limit adverse effects, ziconotide is administered intrathecally during anaesthesia, but its action within the CNS following recovery from anaesthesia can still generate dizziness, nausea, headache and confusion. Because of this, ziconotide is mainly given to patients with late-stage cancer for analgesia in palliative care.

1.1.3.3 Glial environment within the dorsal horn

Resident glial cells (astrocytes, oligodendrocytes and microglia) and immigrant T-cells and macrophages infiltrate the dorsal horn following damage to the spinal cord or first-order

nociceptive fibres, with subsequent loss of integrity of the blood–CNS barrier. The release of cytokines, excitatory amino acids, neurotrophic factors and prostaglandins (PGs) by microglia can cause hyperexcitability of dorsal horn sensory neurons and central sensitization (Gwak, Kang et al. 2012). Dysfunctional glial cells are key contributors in underlying cellular mechanisms contributing to neuropathic pain (gliopathy).

1.1.3.3.i Pharmacological application

Ionic imbalances, neurogenic inflammation and alterations of cell cycle proteins are the predominant neuroanatomical and neurochemical changes that result in glial cell activation and gliopathy. Neuromodulators (anticonvulsants, cannabinoids, gabapentinoids) and anti-inflammatory approaches targeting cytokine release and/or activity can mitigate microglial activation. Anticonvulsants limit the activation of peroxisome proliferator-activated receptors (PPARs) and control microglial activation. Microglial activation can also be decreased by fatty acid-based therapy (*n*-3 polyunsaturated fatty acids, oleic acid, valproic acid), which reduces the release of proinflammatory cytokines involved in PPAR activation, also inducing blockade of voltage-gated sodium channels and a GABAergic effect (Avila-Martin, Galan-Arriero et al. 2011, Fandel, Wasmuht et al. 2013, Lim, Huang et al. 2010).

1.1.3.4 Spinocerebral pathways and supraspinal centres

The spinothalamic tract transmits nociceptive information from the dorsal horn of the spinal cord to the brain. Its lateral aspect projects both contralaterally and ipsilaterally to the lateral thalamus and transmits sensory and discriminative information associated with sharp and short-lasting pain. Its medial aspect projects contralaterally to the medial thalamus and is associated with poorly localized, persistent and diffuse pain, the emotional and aversive aspects of pain, and arousal, motivation and motor responses (Lima 2009). The spinothalamic pathway is the major ascending nociceptive pathway in rodents and primates, but is thought to be less important in carnivores, especially with respect to the spinocervicothalamic tract (Shilo and Pascoe 2014).

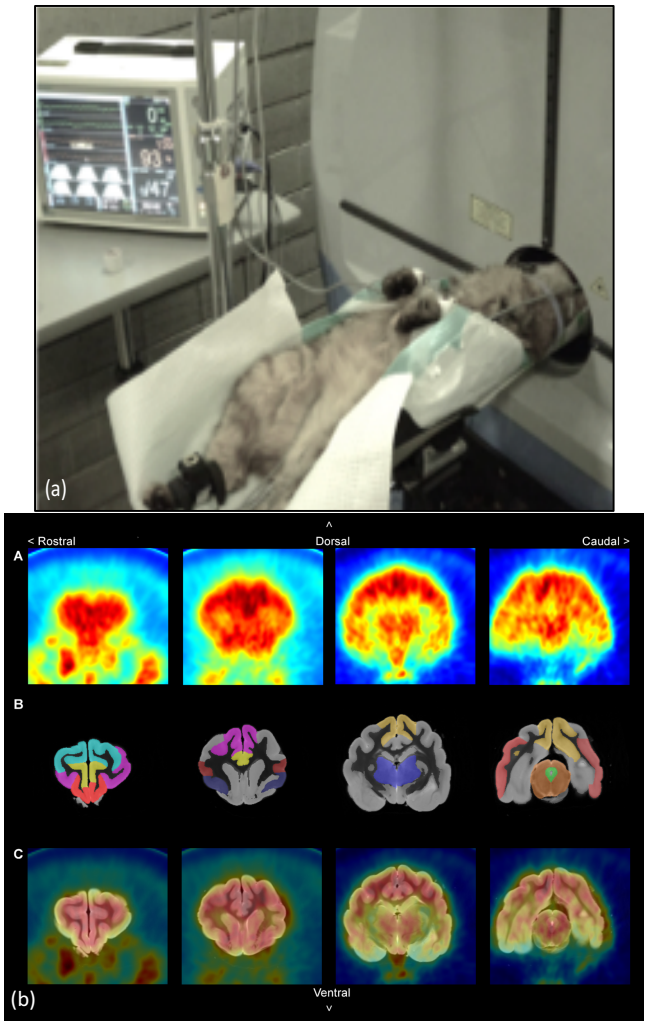
There is some degree of GABA-mediated inhibition of nociceptive transmission in the thalamus. Altered processing of pathways within the thalamus results in the development of a 'thalamo-cortical dysrhythmia', which is recognized as a source of neuropathic pain (Henderson, Peck et al. 2013). From the thalamus, nociceptive signals are transmitted to various areas of the brain, including the primary (SI) and secondary (SII) somatosensory cortices, the insular cortex, the anterior (ACG), mid- and posterior cingulate gyrus, the basal ganglia and the frontal motor cortex.

There are also spinal projections that transmit nociceptive information to other parts of the brain, such as the reticular formation, the medulla (including the nucleus of the solitary tract), the pons (including the parabrachial nuclei), the periaqueductal grey (PAG), the hypothalamus, the basal ganglia, the amygdala and the cerebral cortex.

Nociceptive signal transmission therefore occurs *via* multiple routes, either directly or *via* multi-synaptic relays. The multitude of brain areas involved in nociceptive processing produces the many aspects of pain, which include sensation and sensory discrimination (SI and SII sensory cortices, insula, lateral thalamus), affective-motivational (emotional) and evaluative-cognitive (learning) effects (amygdala, ACG, mid-cingulate gyrus, insula, basal ganglia), motor responses (motor cortex, basal ganglia), changes in arousal (reticular formation) and autonomic responses (hypothalamus, pons) (Davis and Moayedi 2013).

Activation in and around SII and the insula are of particular interest, because increased brain metabolism in the SII cortex of cats with OA has been reported (Guillot, Chartrand et al. 2015) (Figure 1.1.7). These regions are the most strongly activated in response to noxious and innocuous stimuli in neuropathic models compared with controls (Saab 2012). Greater stimulation was also observed at the level of the thalamus and PAG areas in cats with OA, suggesting the involvement of descending modulatory systems in osteoarthritic cats with chronic pain (Guillot, Chartrand et al. 2015). In addition, electroencephalography has revealed dysfunctional networks in patients that are in pain (Saab 2012), and the authors of this chapter have found a significantly higher resting electroencephalographic spectral power in cats with OA compared with healthy cats.

Figure 1.1.7: Brain metabolism in osteoarthritic cats.



Legend: (a) Increased brain metabolism in the SII cortex as well as thalamus and PAG of osteoarthritic cats is illustrated in transverse sections of the brain during positive emission tomography/magnetic resonance imaging techniques (Guillot, Chartrand et al. 2015). (b) Four transversal slices of: (A) an osteoarthritic cat brain imaged with [18F)-fluorodeoxyglucose using a small animal positron emission tomography (PET) scanner; (B) brain regions of interest (ROI) segmented from magnetic resonance (MR) images; (C) PET signal co-registered with MR images. ROI identification from left to right: Slice 1: *salmon*, prefrontal cortex; *aqua*, motor cortex; *purple*, primary somatosensory cortex; *yellow*, anterior cingulate

cortex. Slice 2: *purple*, primary somatosensory cortex; *yellow*, anterior cingulate cortex; *dark blue*, insula; *dark red*, secondary somatosensory (SII) cortex. Slice 3: *blue*, thalamus; *dark yellow*, visual cortex. Slice 4: *dark yellow*, visual cortex; *green*, periaqueductal gray (PAG) matter; *orange*, mesencephalon; *light red*, superior temporal cortex.

1.1.3.5 Descending pathways and inhibition

Descending fibres influence pain processing and perception in response to a given stimulus, depending on various factors, such as emotional state (*e.g.* fear, anxiety) and learning. This produces differences in pain experiences for a particular stimulus. The descending modulation of spinal nociceptive processing can be either inhibitory (antinociceptive, endogenous analgesia), for example, for urgent fight-or-flight responses, or facilitatory (pronociceptive). Although various areas of the brain are involved in descending pain modulation, pathways originating in the midbrain are of particular importance; in particular, the PAG and rostroventral medulla (RVM) axis can either inhibit or facilitate dorsal horn pain processing.

The descending modulatory system receives input from the ACG, the anterior insular cortex and the amygdala, allowing influence by affective-motivational and evaluative-cognitive processes. The PAG of the midbrain has descending inhibitory pathways, which end in enkephalinergic neurons at each spinal segment, producing inhibition of interneurons stimulated by first-order nociceptive fibres. Inhibitory control from the PAG-RVM system preferentially suppresses nociceptive inputs mediated through C fibres, preserving sensory-discriminative information through sensory A fibres.

Adrenergic and serotonergic pathways descending from the locus coeruleus and nucleus raphe magnus in the brainstem can also activate enkephalinergic neurons in the dorsal horn. There are also (ascending) projections of dopaminergic nociceptive neurons from the substantia nigra in the midbrain to the basal nuclei; dopamine has an analgesic effect in chronic pain. Inhibitory fibres can also be found segmentally in the spinal cord (Woolf and Mannion 1999), and endogenous opioids (*e.g.* endorphins, dynorphins and endomorphins) can

also be released concurrently with excitatory neurotransmitters (*e.g*. glutamate) and act on mu, delta and kappa opioid receptors to mediate analgesia. In addition to endogenous opioids, noradrenaline, GABA, serotonin and dopamine, other neurotransmitters involved in antinociceptive pathways include adenosine, somatostatin and cannabinoids.

A commonly used protocol for pain inhibition is based on the diffuse noxious inhibitory control effect, recently renamed conditioned pain modulation. This typically uses two remote painful stimuli, whose interaction generates, in most cases, inhibition of pain (Le Bars, Dickenson et al. 1979). Practical applications in veterinary medicine include the use of nose tongs in cattle, a twitch placed on a horse's upper lip or pinching a skin fold on the neck.

1.1.3.5.i Pharmacological application

The reinforcement of endogenous inhibitory descending modulation using opioid and alpha-2 adrenergic agonists is a popular target for analgesia. Drugs in development include GABA and synthetic cannabinoid agonists. Additional medications found to have central analgesic effects in humans include those with serotonergic and noradrenergic activity, such as tricyclic antidepressants (*e.g.* amitriptyline), serotonin–noradrenaline reuptake inhibitors (*e.g.* duloxetine) and, to a lesser extent, selective serotonin reuptake inhibitors. There is some evidence that these drugs may also act by blocking voltage-gated sodium channels. Tramadol is an example of an analgesic agent that combines opioid, serotonergic and noradrenergic activity.

1.1.4 Altered pain states

Normal pain processing is the result of a carefully maintained equilibrium. When changes occur within nerves or in their environment, that equilibrium is disrupted, producing sensory changes and abnormal pain conditions. Injury to the nervous system can cause both increased and decreased activity, resulting in sensory deficits or loss of sensation, hypersensitivity states, spontaneous pain, and other abnormal sensations.

1.1.4.1 Signs associated with altered pain states

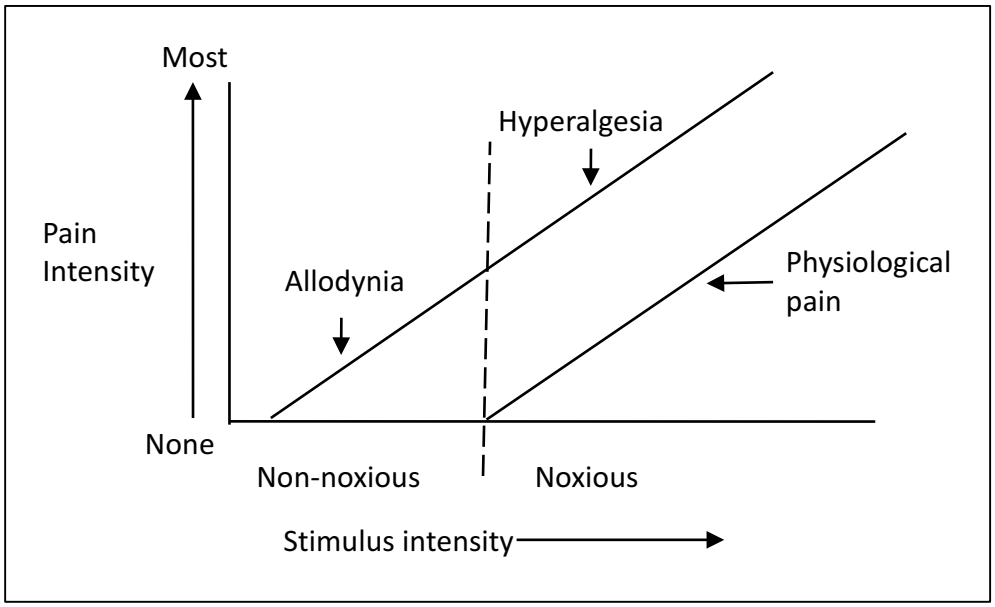
1.1.4.1.i Allodynia

This is the sensation of pain in response to a normally innocuous stimulus (Figures 1.1.8 and 1.1.9). Allodynia may result either from a lowered threshold of nociceptive terminals (as occurs in peripheral sensitization) or from the activation of low-threshold Aβ (sensory) fibres following central sensitization. An example of allodynia is pain in response to light touch, as demonstrated by the decreased tactile threshold observed in approximately 30% of cats with OA (Guillot, Moreau et al. 2013).

1.1.4.1.ii Hyperalgesia

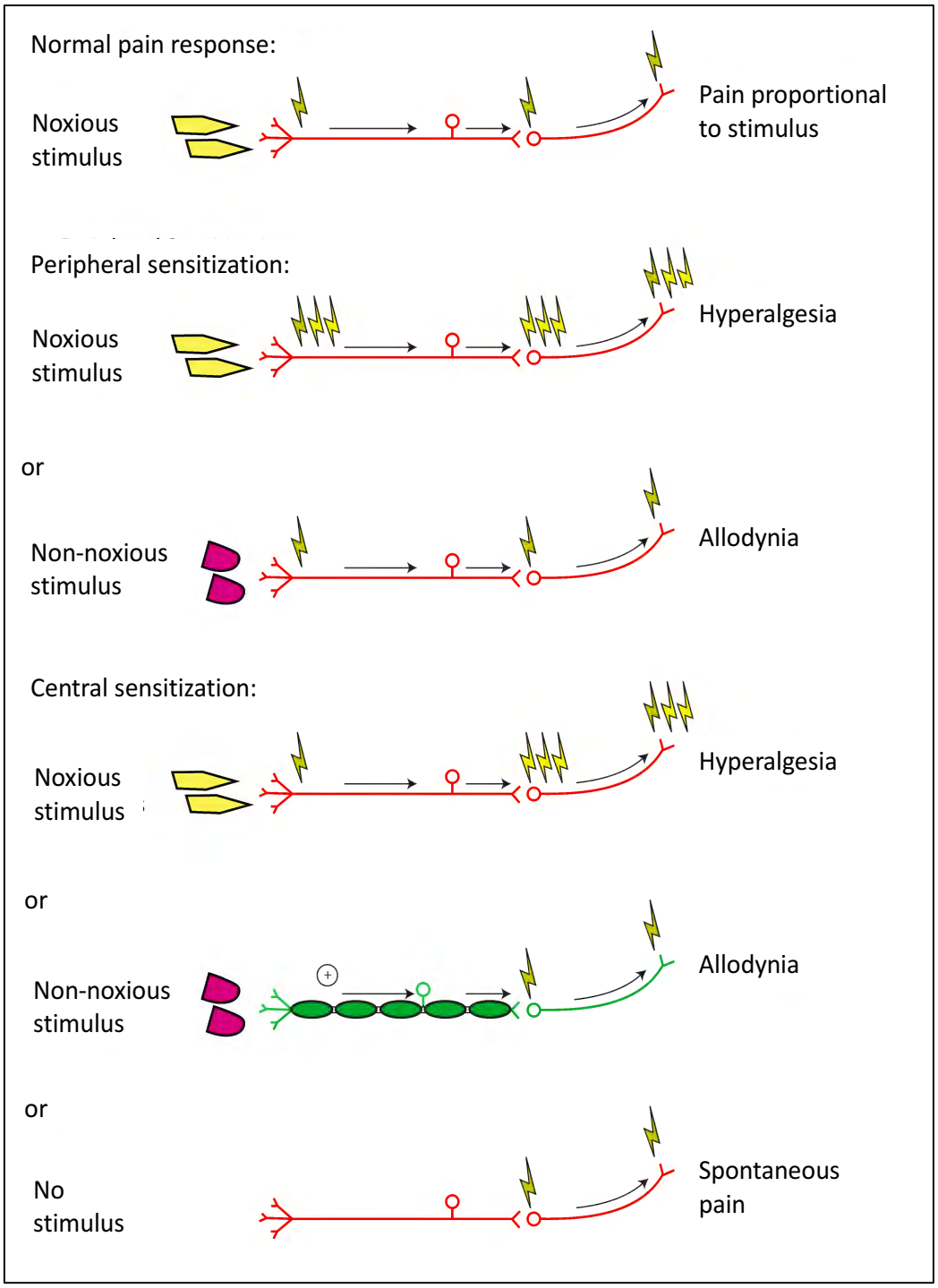
This refers to an exaggerated pain sensation in response to a normally painful stimulus (Figures 1.1.8 and 1.1.9). Hyperalgesia can be classified on the basis of the modality (mechanical, thermal or chemical); in humans, mechanical hyperalgesia is subdivided into either dynamic (evoked by brushing) or static/punctate (evoked by pressure) hyperalgesia. Dynamic hyperalgesia results from central pain responses to Aβ fibre stimulation. An example of hyperalgesia is exaggerated pain in response to a slightly painful procedure such as a skin pinch.

Figure 1.1.8: Graph illustrating various pain states.



Legend: See text for further details.

Figure 1.1.9: Diagram illustrating the changes that cause allodynia, hyperalgesia and spontaneous pain.



(© Juliane Deubner, University of Saskatchewan, Canada)

1.1.4.1.ii.a Primary hyperalgesia

Primary hyperalgesia is associated with nociceptor sensitization in the region of an injury, where the nociceptive terminals are exposed to inflammatory mediators. An example of this is the pain felt following surgery in the area of the incision and in the surrounding swelling/bruising.

1.1.4.1.ii.b Secondary hyperalgesia

This refers to hypersensitivity that cannot be explained by sensitization of peripheral nociceptor terminals, because it arises adjacent to, but outside (or even contralateral to), the area where inflammation/injury is present. It provides evidence of central sensitization. An example of secondary hyperalgesia is increased sensitivity to claw trimming in the hindlimb of a dog with hip OA.

1.1.4.1.iii Spontaneous pain

As the name implies, spontaneous pain is pain that arises in the absence of a stimulus. This is reported in humans with neuropathic pain, but may be difficult to identify in animals because they are unable to self-report their experience of pain. It may also be difficult to distinguish from paraesthesia or dysaesthesia (see below). A possible manifestation of spontaneous pain in animals is sudden attention to a body part, for example, a tail-docked dog abruptly nibbling at its healed tail stump.

1.1.4.1.iv Paraesthesia

This refers to non-painful but abnormal sensations, sometimes described in humans as tickling or tingling. These sensations may result from spontaneous activity in Aβ (sensory) fibres.

1.1.4.1.v Dysaesthesia

This refers to unpleasant abnormal sensations that may or may not be painful; examples of such sensations reported by humans are burning, shocks, or 'pins and needles'. Dysaesthesia may develop from paraesthesia when central sensitization occurs.

1.1.4.1.vi Analgesia

This refers to the absence of pain in response to a normally painful stimulus. By extension, it has been recognized as the treatment of pain, either before (pre-emptive or preventive) or after (curative) it occurs.

1.1.4.1.vii Anaesthesia

This refers to an absence of any sensation in response to a stimulus. An example of this is the lack of response to various intensities of hindlimb toe stimulation in a dog with a spinal injury.

1.1.4.1.ix Hypoalgesia

This refers to decreased pain sensation in response to a painful stimulus.

1.1.4.1.x Hypoaesthesia

This refers to decreased sensation in response to a stimulus.

1.1.5 Sensitization

Sensitization is defined as a decrease in the threshold and an increase in the magnitude of the response to noxious stimulation. Responsiveness to previously non-noxious stimuli and spontaneous nociceptive signal transmission may both develop.

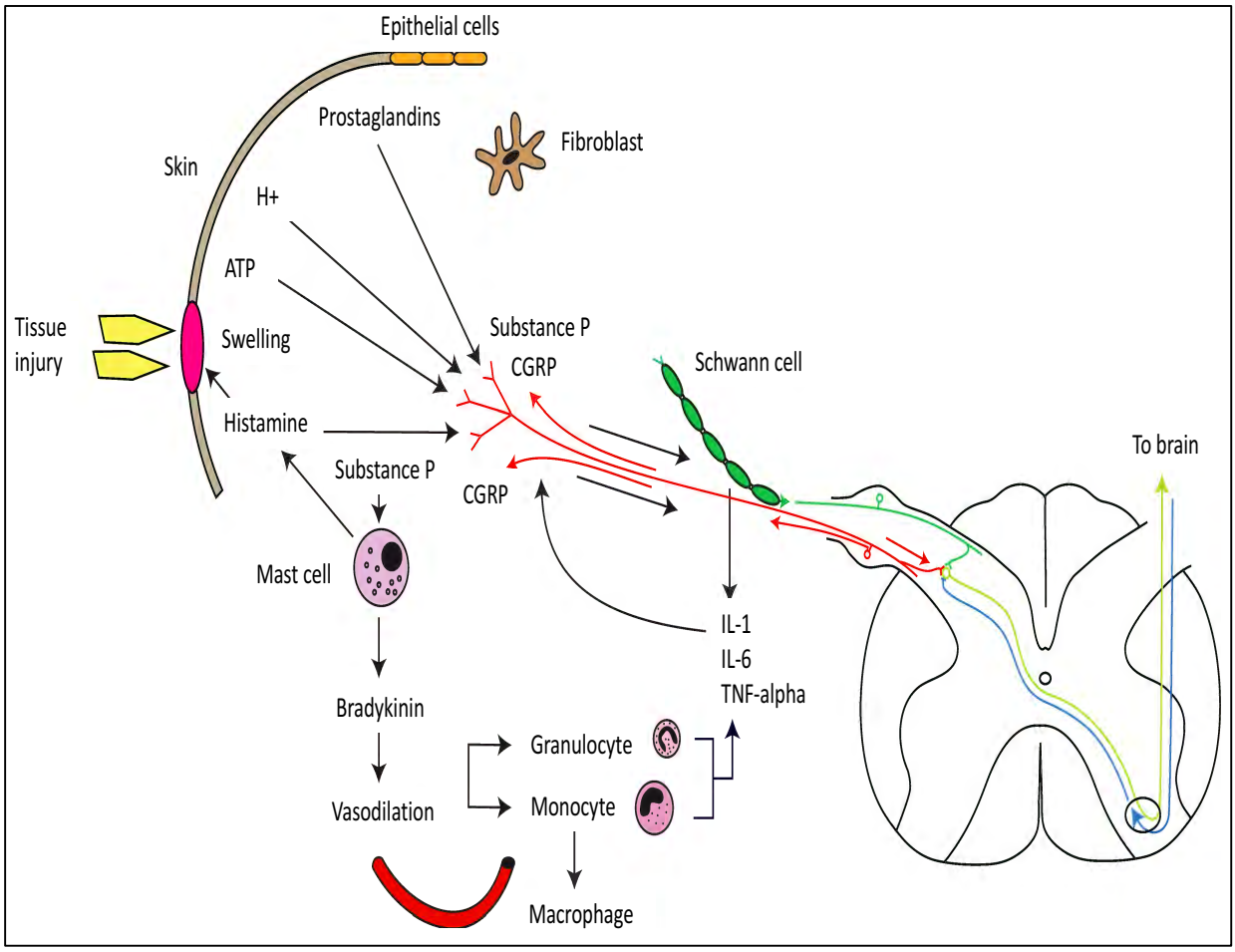
1.1.5.1 Peripheral sensitization

Peripheral sensitization is associated with a reduction in the activation threshold and an increase in the responsiveness of peripheral nociceptor terminals (Figure 1.1.10). The mechanisms by which nociceptors are sensitized include:

- Tissue inflammation secondary to injury, infection, *etc.*, causing changes in the chemical environment of the nociceptor
- Changes in the nociceptive neuron itself, resulting from injury (*e.g.* altered expression of ion channels)
- Neurogenic inflammation, in which a nociceptive neuron secretes inflammatory substances into its own environment.

Nerve growth factors are implicated in peripheral sensitization states, in that loss of access to these trophic factors after peripheral nerve injury, or their increased production secondary to peripheral inflammation, can increase nociceptor excitability.

Figure 1.1.10: Diagram showing the action of inflammatory mediators on nociceptors and peripheral sensitization.



Legend: ATP = adenosine triphosphate; CGRP = calcitonin gene-related peptide; IL = interleukin; TNF = tumour necrosis factor. (© Juliane Deubner, University of Saskatchewan, Canada)

1.1.5.1.i Neurogenic inflammation

Peptidergic nociceptive first-order neurons contain substance P and/or CGRP and can release these substances from their peripheral terminals (*via* antidromic transmission). Both of these substances act directly on vascular endothelial and smooth muscle cells to produce vasodilation and increased capillary permeability. These effects normally contribute to tissue

homeostasis, but in injury or sterile inflammation they can produce plasma extravasation and oedema. Both neurotransmitters also sensitize the terminals of injured and adjacent nerves. Nerve growth factor contributes to neurogenic inflammation by promoting increased production of substance P and CGRP in nociceptor neurons.

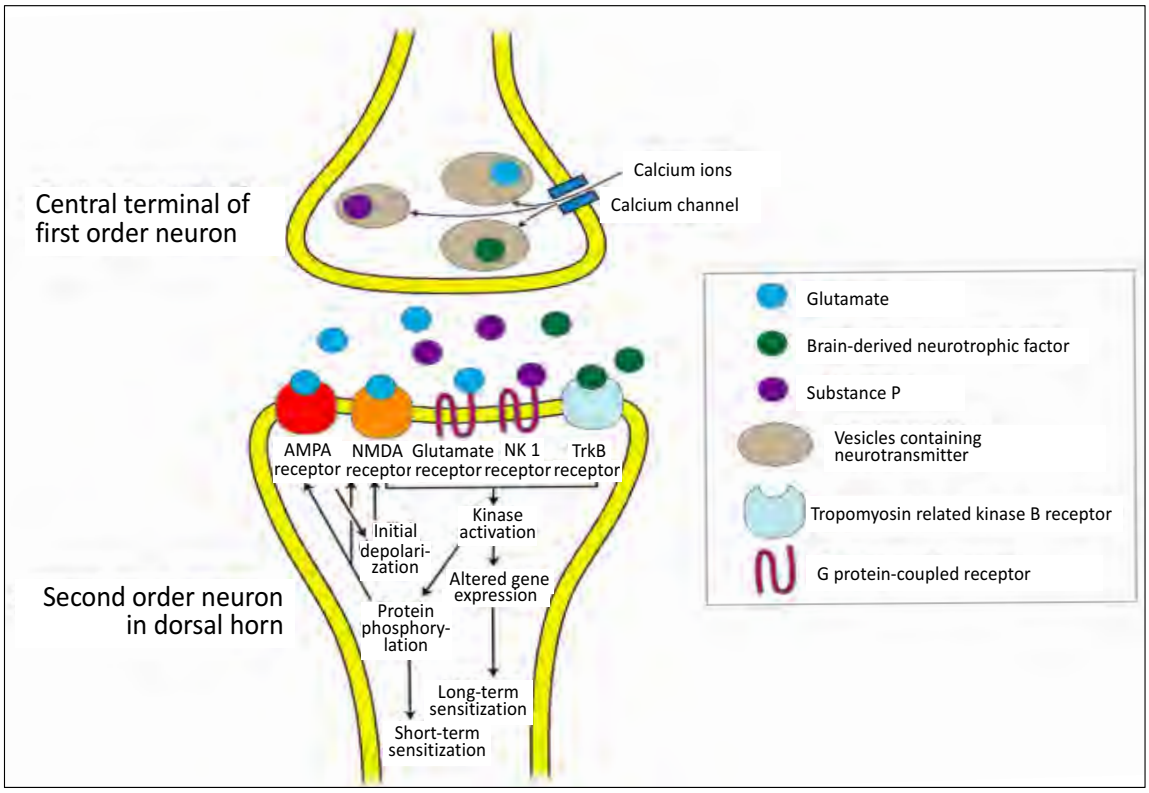
Nociceptors can release other substances that may also contribute to neurogenic inflammation, including ATP, adrenomedullin, neurokinins, vasoactive intestinal polypeptide (VIP), neuropeptide Y, gastrin-releasing peptide, glutamate, nitric oxide (NO) and cytokines. In addition to vasodilation and plasma extravasation, their release results in the attraction and activation of immune cells; neuropeptides, chemokines and glutamate are chemotactic for neutrophils, eosinophils, macrophages and T cells.

The resulting inflammation sensitizes peripheral nociceptors (see section on inflammatory pain), which then release more immune factors, creating a positive feedback loop. This means that the immune system activation and nociceptor (peripheral) sensitization that occur after injury create the required conditions to prime nociceptive processing, which will lead to central sensitization.

1.1.5.2 Central sensitization

Central sensitization arises from an increased efficiency of pain signal transmission by nociceptive pathways and can persist following the cessation of nociceptor signalling. It results from intense, prolonged and/or repeated nociceptive input, which may be due to peripheral tissue injury, peripheral nerve injury, or non-injurious noxious stimuli (Figure 1.1.11). This leads to changes in membrane excitability, synaptic efficacy and/or reductions in inhibition (Latremoliere and Woolf 2009). Central sensitization therefore represents an uncoupling of the clear stimulus-response relationship with pain, such that, at the extreme, a noxious stimulus may not be necessary for pain to occur. Some individuals appear to be more susceptible to central sensitization than others, and environmental and genetic factors probably contribute to differences between individuals' pain sensitivity and responsiveness to treatment.

Figure 1.1.11: Central sensitization produces changes within the terminals of the neurons to ensure that nociceptive transmission occurs.



Legend: See text for further details. AMPA = α-amino-hydroxy-5-methyl-4-isoxazolepropionic acid; NK-1 = neurokinin-1; NMDA = *N*-methyl-$_D$-aspartate. (© Juliane Deubner, University of Saskatchewan, Canada)

Delays in recognition that central sensitization may be responsible for pain in the absence of observable pathology have led to human patients who claimed to be in pain not being taken seriously (Woolf 2011). This problem is relevant to veterinary surgeons (veterinarians) because animals that respond in an exaggerated manner to relatively benign handling and non-painful procedures may sometimes do so because of this exaggerated processing of pain.

Repeated or prolonged nociceptive input from chronic inflammation (*e.g.* OA) or due to peripheral nerve injury (resulting in increased nociceptor sensitivity or spontaneous activity) will cause central sensitization. After peripheral nerve injury, spinal microglia are

activated and accumulate in the dorsal horn around the terminals of the injured neurons; there, they release inflammatory mediators, which further enhance sensitization (Ren and Dubner 2010). Persistent nociceptive input leads to the activation of protein kinases and phosphorylation of the NMDA receptor, which results in intracellular calcium accumulation. As a consequence, there is increased secretion of excitatory neurotransmitters by nociceptive neurons and increased responsiveness of connecting dorsal horn cells to these neurotransmitters, possibly *via* increases in dendritic spines, which ensures long-lasting dorsal horn nociceptive transmission (Xu and Yaksh 2011).

Many parallel signal inputs to dorsal horn neurons can contribute to the initiation of central sensitization, either separately or cooperatively. Dorsal horn neurons normally receive innocuous small-amplitude inputs from low-threshold sensory (Aβ) neurons and from nociceptors outside their receptive fields, in addition to large-amplitude nociceptive inputs. When these low-threshold inputs become capable of activating a dorsal horn neuron, the sensation of pain can be induced by non-nociceptive stimuli, and alterations in receptive fields can develop. In addition, spontaneous activity and temporal summation ('wind-up') of stimuli that would otherwise be subthreshold can occur, so that that repetition of such stimuli generates an increasingly intense response. Once central sensitization has been established, it can then be maintained by a lower-level nociceptive input or by different kinds of non-nociceptive inputs other than those that caused the initial development of central sensitization.

The increased excitability of spinal cord neurons produces heightened clinical pain sensitivity, manifested by a reduced threshold for pain (allodynia), an increased strength and duration of the response to painful stimuli (hyperalgesia), ongoing transmission of pain signals after a stimulus is terminated (after-discharges), and increased peripheral receptor field size, beyond the area of the affected nerve. All of these changes mean that input from neighbouring uninjured tissue can produce pain (secondary hyperalgesia). Patients with central sensitization have lower thermal and mechanical thresholds in a diffuse pattern, which reflects the enlargement of the spinal cord neuron receptive fields. There is a change in nociceptive-specific neurons to become *convergent neurons* – that is, they begin to respond to both innocuous and noxious stimuli. Low-frequency repetition of a fixed-intensity stimulus

increases the action potential discharge of dorsal horn neurons followed by after-discharges. This activity-dependent facilitation is called spinal wind-up and, in the presence of spinal plasticity, is associated with temporal and/or spatial summation. Therefore, repeated stimulation results in painful after-sensations that persist after the stimulus is withdrawn, or the rating for the pain (*i.e.* its intensity) for the last stimulus is greater than the pain rating for the first stimulus, even though the stimuli are exactly the same. There may also be an extension of the receptive field.

Central sensitization also has supraspinal components (Schaible 2012). For example, microglia in the brainstem are activated after peripheral nerve injury, contributing to supraspinal facilitation of pain signalling. Imaging studies in humans with chronic pain have shown structural changes in various brain regions, although the cause–effect relationship of such changes with pain remains unclear. For example, modifications have been demonstrated in grey matter volume in the prefrontal cortex, insula, ACG and mid-cingulate gyrus, as well as in the thalamus, basal ganglia, SI and SII somatosensory cortices and brainstem; some of these changes resolve following successful pain management. Changes in white matter have also been demonstrated in some pain states, suggesting that in chronic pain there are changes in communication between different areas of the brain.

1.1.5.2.i Possible mechanisms of central sensitization

Some of the mechanisms implicated in central sensitization include altered glutamatergic neurotransmission/NMDA receptor-mediated hypersensitivity, loss of tonic inhibitory controls, and glial–neuronal interactions. Glutamate, the main excitatory neurotransmitter released by first-order nociceptive neurons, binds to postsynaptic AMPA and kainate ionotropic receptors, causing membrane depolarization. Glutamate also binds to metabotropic glutamate receptors, as well as the normally silent NMDA receptors. The NMDA receptors are activated *via* a complex cascade of events. Increased AMPA receptor production, phosphorylation (activation) of membrane channels and receptors by tyrosine kinases and activation of intracellular enzymes (*e.g.* phospholipase A_2) enhance NMDA receptor activity. Full activation of NMDA glutamate receptors also requires the binding of glycine and displacement of a magnesium ion from the calcium channel of this ionotropic

receptor; the latter occurs when either substance P, CGRP or AMPA binds to its own receptor on the same membrane. Activation of AMPA/NMDA receptors leads to an increase in intracellular calcium, which results in an increased strength of synaptic connections between nociceptors and dorsal horn neurons.

Substance P and CGRP are involved in transmission between nociceptors and the CNS, and also play a role in central sensitization. Substance P binds to the metabotropic NK-1 receptor on second-order neurons, causing long-lasting depolarization. The effect of substance P is potentiated by CGRP receptor stimulation. Synthesis and release of brain-derived neurotrophic factor from nociceptive neurons stimulates tropomyosin-related kinase B receptors, which further activates protein kinases (see Figure 1.1.11). Wind-up results from activation of NK-1 and CGRP receptors, which permit repeated low-frequency stimuli to produce a cumulative membrane depolarization. Wind-up itself generally lasts only a few seconds.

Bradykinin and serotonin (*via* 5-HT3 receptors) contribute to central sensitization by increasing synaptic strength, and NO also contributes by activating guanylate cyclase, resulting in increased neuronal excitability and decreased inhibition.

Glycinergic and GABAergic interneurons normally inhibit nociceptive transmission, but this inhibition is lost, possibly through mechanisms including GABAergic neuronal cell death and alterations in potassium–chloride co-transporters, so that GABA receptor activation now depolarizes rather than hyperpolarizes the cell membrane. PGE_2 also acts on spinal excitatory interneurons and projection neurons to cause phosphorylation of glycine receptors, rendering the neurons unresponsive to the inhibitory effects of glycine.

Some forms of activity-dependent plasticity are very brief, others are relatively long-lasting and involve changes in protein phosphorylation and altered gene expression, and some changes are irreversible, with loss of neurons and creation of neuronal sprouting (the formation of new synapses).

With central sensitization, the main therapeutic aim is to block NMDA receptors with the use of drugs such as ketamine, dextromethorphan, amantadine or methadone. Other therapeutic agents include glycine and NK-1 receptor antagonists and inhibitors of neuronal NO synthase or protein kinase. Decreasing presynaptic calcium conductance by administration of gabapentinoids, cannabinoids, opioids or alpha-2 adrenergic agonists reduces neurotransmitter release, thus reducing the transmission of nociceptive information to, and within, the spinal cord neurons and re-enforcing endogenous inhibition.

1.1.6 Inflammatory pain

Pain and hypersensitivity resulting from tissue injury are part of a normal protective response. They prevent further damage to an injured area, and promote wound repair by preventing any interference with healing (*e.g.* by causing the animal to immobilize, and prevent contact with, the affected area). Sensitization of nociceptors is usually reversible; it is normal to have increased nociceptor sensitivity after tissue injury, but this should resolve with healing.

The chemical environment of nociceptor terminals determines their baseline sensitivity and threshold for the generation of action potentials. When tissues are injured, inflammation develops and normally persists until the tissues have healed. Inflammation can alter the chemical environment of the nociceptor, producing a lower threshold for activation, that is, increased sensitivity of both the affected area and adjacent nociceptors exposed to the same chemical changes (see Figure 1.1.10). Sympathetic postganglionic neurons, Schwann cells, mast cells, basophils, platelets, macrophages, neutrophils, endothelial cells, keratinocytes and fibroblasts also produce mediators that can act on nociceptive neurons following tissue injury.

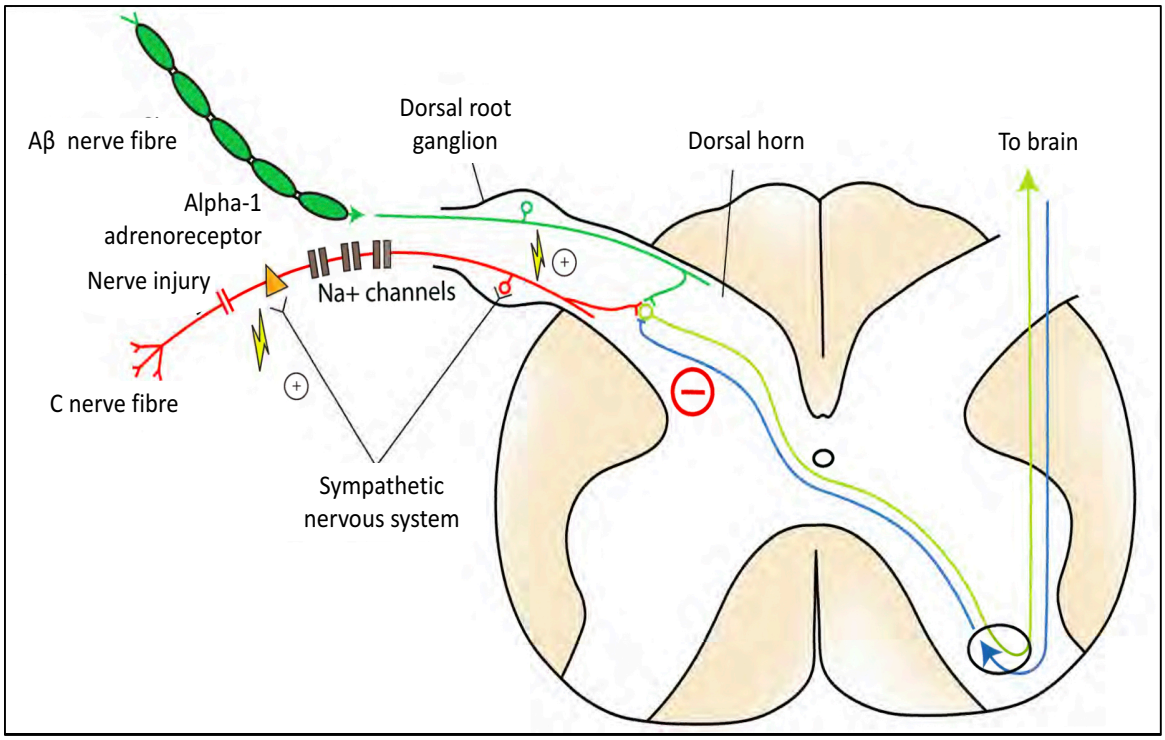
Inflammatory cytokines including nuclear factor-κB, interleukins (IL-1β, IL-6) and tumour necrosis factor-α (TNF-α) act directly on nociceptors, and promote further inflammation and the production of pro-algesic compounds such as PGs, NGF, bradykinin, and extracellular protons. Substance P and CGRP are both locally released by antidromic activation (the ‘local axon reflex’). Other molecules involved in inflammation (serotonin, eicosanoids, thromboxanes, leukotrienes, endocannabinoids, chemokines and extracellular

proteases) and mast cell degranulation (histamine, bradykinin) can also sensitize nociceptors. Some inflammatory mediators directly alter neuronal excitability by interacting with membrane ion channels, whereas others (*e.g.* bradykinin and NGF) act indirectly *via* metabotropic receptors and secondary messenger cascades. NGF is produced by various cells at inflammatory sites; it alters the expression of membrane channels and receptors such as TRPV1 and voltage-gated sodium channels, and increases the production of substance P and CGRP in DRG neurons.

1.1.7 Neuropathic pain

Neuropathic pain is associated with injury or disease affecting parts of the nervous system, that is, peripheral nerves, DRG or the dorsal root, or the CNS (Mathews 2008) (Figure 1.1.12). Some examples of conditions that can produce neuropathic pain in small animals include diabetic neuropathy, amputation or other surgical/traumatic nerve injury and neoplasia. Whereas inflammatory pain may be relieved by eliminating the stimuli that are affecting the inflamed tissue, neuropathic pain may be ongoing. Following nerve injury, a number of changes can occur and may contribute to chronic neuropathic pain: these include changes in the injured nerve itself, in the surrounding nociceptive, sensory and sympathetic nerves, and in glial and Schwann cells.

Figure 1.1.12: Neuropathic pain originates from nerve damage and local changes such as increased sympathetic activity and input from Aß fibres.



Legend: There is less descending inhibition of nociceptive transmission. A∂ fibres not shown for clarity. (© Juliane Deubner, University of Saskatchewan, Canada)

There may be modifications in the presence, numbers or types of nociceptive neuronal transducers, which include ionotropic and metabotropic receptors. For example, mechanical and/or thermal transducers may appear at or near the cut ends of damaged axons or within the ganglia; certain transducers may be expressed in neurons that do not normally have them; there may be decreases in inhibitory (*e.g.* opioid) receptors and increases in excitatory (*e.g.* purinergic) receptors; and there may be changes in the coupling between transducers and signalling pathways. There may also be changes in the expression and/or release of ligands and receptors, and aberrant sources of nociceptor activation may develop.

When a sensory neuron is injured, the number of sodium channels increases both at the site of injury and along the axon and cell body. This results in foci of hypersensitivity and

ectopic foci, producing increases in stimulus-evoked pain and in spontaneous pain. There are two main types of sodium channels, tetrodotoxin-sensitive and tetrodotoxin-insensitive, with the insensitive channels normally being found only in nociceptive neurons; however, both types of sodium channels develop in sensory nerves after they have been injured. Schwann cells can also release TNF-α after nerve injury, which then produces inflammation. Demyelination resulting from injury can also cause hyperexcitability in neurons. In addition, peripheral nerve injury can induce sprouting of Aβ fibres from their usual location in laminae III and IV into laminae I and II of the dorsal horn, which normally receive only C-fibre input. Although C and Aδ fibres may downregulate production of substance P and CGRP following peripheral nerve injury, Aβ fibres begin to produce them, so normally innocuous stimuli can cause release of these neurotransmitters. All of the above changes may result in the interpretation of normally innocuous sensations as painful (allodynia).

Inflammation within a nerve or ganglion can result in alterations to the function and chemistry of a nerve. Inflammation can increase NO because inducible NO synthase is present in neurons and glial cells in the dorsal horn of the spinal cord. NO can sensitize dorsal horn neurons, increase excitatory neurotransmitter output by first-order nociceptive neurons, and increase PG and cytokine production by non-neuronal cells. In addition, PGs released in inflammation (PGE_2) can decrease the inhibitory action of glycine, resulting in increased pain sensation.

Neuropathic pain can be associated with an increase in sympathetic nervous system activity. Alpha-1 adrenergic receptors develop following injury in both affected and adjacent uninjured nociceptive neurons; alpha-2 adrenergic receptors change from being coupled to inhibitory second messenger pathways to being coupled to excitatory pathways, permitting sympathetic nervous system stimulation of nociceptive fibres. Sympathetic axons also sprout in a basket shape around the cell bodies of injured sensory neurons in the DRG.

Peripheral nerve injury can also produce disinhibition of dorsal horn neurons *via* various mechanisms. Both GABA and opioid receptors are downregulated following injury to afferent nociceptive fibres. In addition, synthesis of GABA and glycine may be decreased, production of cholecystokinin (which inhibits opioid receptors) may be increased and

inhibitory interneurons may die off, thus altering the normal balance between excitation and inhibition. The result of the decreased inhibitory activity is increased pain sensation.

Neuropathic pain is also associated with decreased thalamic reticular nucleus and SI somatosensory cortex activity, decreased thalamic GABA content and altered functional connectivity between the somatosensory thalamus and various cortical regions associated with pain processing.

1.1.8 Therapeutic targets in the pathophysiology of pain

Analgesic drugs may act at any level in the pain pathway, but an understanding of their mechanisms and sites of action helps to guide selection of an appropriate drug for a given condition. For example, the use of TRPV1 blockade is emerging as a method to stop the transduction and conduction of nociceptive signals.

Local anaesthetics (*e.g.* lidocaine) provide analgesia by blocking voltage-gated sodium channels, preventing action potential generation in sensory neurons and therefore preventing nociceptive signal transmission from the periphery (see Chapter 11). When administered systemically at low doses, they may also block NMDA receptors in the dorsal horn of the spinal cord, thereby alleviating neuropathic pain (Mathews 2008). Other compounds target ion channels, such as specific sodium, potassium or calcium channels. In particular, gabapentinoids (gabapentin, pregabalin) that bind to the α2δ1 subunit of N-type voltage-dependent calcium channels have been shown to prevent central sensitization (Phillips and Clauw 2013, Woolf 2011).

Anti-inflammatory medications include corticosteroids (*e.g.* prednisolone) and nonsteroidal anti-inflammatory drugs (NSAIDs; *e.g.* carprofen, meloxicam, coxibs). The NSAIDs act by inhibiting cyclo-oxygenases (COX) and block the conversion of arachidonic acid into proinflammatory prostanoids such as PGE_2 (see Chapter 10), which sensitize nociceptor terminals *via* binding to metabotropic receptors (Julius and Basbaum 2001). Their use addresses the inflammatory aspect of pain. Removal of inflammatory mediators from affected body parts normalizes the environment surrounding nociceptor terminals, thereby

returning their threshold for activation to normal levels. However, COX products are also present in the spinal cord and may interact with the central terminals of nociceptive first-order neurons; for instance, PGE_2 increases the excitability of DRG neurons by altering the activity of tetrodotoxin-resistant sodium channels towards hyperpolarization, and also participates in gliopathy. This suggests that NSAIDs can also have central effects (Schaible 2012).

Neuropathic pain does not respond to NSAIDs (Woolf and Mannion 1999) and COX inhibitors are ineffective for central sensitization, unless it was triggered by peripheral inflammation (Woolf 2011). A related compound, paracetamol (acetaminophen), has analgesic effects that are mediated by inhibitory influences in the dorsal horn, such as reduction of the oxidized form of COX enzymes, interrupting the production of proinflammatory substances, and targeting the cannabinoid pathway (Chiou, Hu et al. 2013). This makes paracetamol useful primarily, but not exclusively, for the treatment of inflammatory pain. Therapeutic blockade of neurogenic inflammation includes the use of anti-cytokines (TNF-α and IL-1β) and NO inhibitors.

Antispasmodic medication (*e.g.* botulinum toxin) has mechanisms of action including interference with protein transport in neurons and decreased release of glutamate, VIP and neuropeptide Y.

Bisphosphonates reduce bone turnover and osteoclast activity, leading to beneficial effects in the treatment of pain in canine osteosarcoma using zoledronate (Fan, de Lorimier et al. 2008) or pamidronate (Fan, Charney et al. 2009), or for canine OA using tiludronate (Moreau, Rialland et al. 2011). The efficacy of tiludronate on pain behaviour and physiological parameters in surgically induced canine OA has been explained by decreased peripheral and central sensitization, as well as by modifications in the release of spinal neuropeptides (Rialland, Otis et al. 2014).

Several analgesic drugs have important central effects. Alpha-2 receptor agonists such as dexmedetomidine produce hyperpolarization of spinal projection neurons and inhibition of neurotransmitter release from primary nociceptive afferents, thereby decreasing pain perception (Meintjes 2012). They show pharmacological synergism with opioid agonists,

resulting in potent analgesia (and sedation), which is particularly useful for the treatment of surgical pain (Chabot-Dore, Schuster et al. 2015).

Mu opioid receptors are most abundant in the PAG of the midbrain and in the substantia gelatinosa of the spinal cord; they act to inhibit neuronal transmission by increasing presynaptic GABA. Both opioid and GABA receptors are found presynaptically on primary sensory neurons and postsynaptically on dorsal horn neurons. There appears to be some functional segregation of opioid receptors at the level of the nociceptor: mu receptors predominate in peptidergic nociceptors, and delta receptors predominate in non-peptidergic receptors. Neuropathic pain may be resistant to opioids (Woolf and Mannion 1999), and opioids may even induce hyperalgesia in some patients (Phillips and Clauw 2011).

Antagonists of NMDA such as ketamine and amantadine block the excitatory effects of glutamate and can block central sensitization, as well as alleviate neuropathic pain (Woolf 2011, Woolf and Mannion 1999). The use of ketamine infusions is common to counteract or prevent central sensitization (see Chapter 10).

Treatments believed to act by restoring endogenous inhibitory systems include drugs that mimic descending or local inhibitory pathways (alpha-2-adrenergic agonists, opioids, tricyclic antidepressants, serotonin and/or noradrenaline reuptake inhibitors, and GABA agonists such as baclofen) (Woolf and Mannion 1999).

Pregabalin and gabapentin are also centrally acting analgesics. Their mechanisms of action are unclear, but may involve blocking of N-type voltage-gated calcium channels (Basbaum, Bautista et al. 2009), presynaptic inhibition of glutamate release (Meintjes 2012) and inhibition of excitatory synaptogenesis (Kuner 2010). These drugs seem to be effective both in reducing central sensitization and in some cases of neuropathic pain (Phillips and Clauw 2013, Woolf 2011).

Non-pharmacological analgesic techniques also exist, including acupuncture, massage, heat and cold therapy, and transcutaneous nerve stimulation. These techniques may provide analgesia in various pain states, including neuropathic pain (Woolf and Mannion 1999), by

activating either segmentary inhibitory control (the gate control theory), and/or descending inhibitory systems (Kuner 2010). Therapeutic diets including *n*-3 polyunsaturated fatty acids may have anti-inflammatory and anti-neuropathic properties, and provide another therapeutic option.

1.1.9 Physiological considerations in pain assessment

An understanding of the physiology of pain processing also permits the manifestations of pain to be explained and predicted to some degree. In humans with chronic pain and some neuropathies, quantitative sensory testing (QST) has been recommended (Backonja, Attal et al. 2013). QST involves measuring and mapping abnormalities of sensation, including hyperalgesia, allodynia, hypoalgesia or analgesia, and the responses to different types of stimuli (*e.g.* brush versus punctate mechanical, hot versus cold). These measurements quantify either altered conditioned pain modulation or exacerbated facilitatory pain processing, or both (Tousignant-Laflamme, Page et al. 2008). Although animals cannot make verbal reports of sensation, some degree of QST is possible and can be used to detect peripheral and central sensitization in conditions associated with pain or sensory loss (Lascelles 2013). For example, central sensitization can be detected using withdrawal reflexes (such as von Frey tests in a laboratory setting) and by identifying allodynia and hyperalgesia in areas that have no demonstrable pathology but surround an injury (Woolf 2011). Examples of conditions in animals with evidence of central sensitization identifiable using QST are foot rot in sheep (Ley, Waterman et al. 1995), OA in cats (Guillot, Moreau et al. 2013), cranial cruciate ligament rupture in dogs (Brydges, Argyle et al. 2012, Rialland, Otis et al. 2014) and ovariohysterectomy in dogs (Lascelles, Cripps et al. 1997).

While QST shows promise as a pain assessment tool, its standardized clinical use has not yet been established in animals. It is also important to consider that QST focuses on the nociceptive (sensory) aspect of pain, and may not reflect the full pain experience of the individual (including affective-motivational and evaluative-cognitive effects) (Brown 2012). Moreover, although conditioned pain modulation can be determined in human patients, it is more difficult to evaluate in animals. However, mechanical temporal summation responses are

faster in cats with OA compared with responses in healthy cats (Guillot, Taylor et al. 2014), and central sensitization (assessed by electrical QST) has demonstrated a clear association with clinical signs, such as kinetics or lameness, in OA-affected dogs (Rialland, Otis et al. 2014).

Pain has emotional, learning and other behavioural aspects; as a result, spontaneous behaviour and behavioural responses to touch and other interactions can be used to assess pain, either in a relatively unstructured manner or by using validated pain scales (see Chapter 9). If pain scales are used, they must be reliable and valid for the particular species, condition and context before being used for the clinical assessment of pain (Streiner and Norman 2008). In human patients with pain, neurological tests can detect changes in the structure and function of the brain compared with individuals without pain; however, practical factors may limit the usefulness of such evaluations in animals.

1.1.10 Conclusion

Pain processing is a complex phenomenon that involves sensory, emotional and learning aspects. Injury, disease or surgery affects tissues and/or nerves and results in altered sensation, including pain, which may or may not outlast the healing process. Central sensitization is an important consideration in the treatment of pain. It is likely to be present in many acute and chronic conditions, whether they were initiated by nerve or tissue injury, and it may manifest itself as painful reactions to contact with apparently uninjured body parts. Central sensitization should be considered in any chronic condition where inflammation or nerve pathology exists, and it may explain differences in the intensity of pain between individuals with comparable detectable pathology (*e.g.* differences in OA pain in the presence of similar joint pathology). Even in animals without obvious injury, abnormalities of sensation may exist. An understanding of the physiological mechanisms involved in different types of pain can help the veterinary surgeon to predict the pain associated with specific conditions and injuries, and to select the most appropriate analgesics. Although evaluation of pain in animals is challenging and lags behind that in humans, partly due to animals' lack of self-reporting, consideration of the sensory, emotional and learning aspects of pain may assist the practitioner

in detecting signs of pain during history taking and physical examination. Standardized assessments including validated pain scales, QST, and possibly even neuroimaging hold promise for the future management of pain.

1.2 Pain Scales

The following section on pain scales contains portions of an unpublished course paper originally written by the doctoral candidate in the context of a graduate class, Pain Management in Children (MCG 9407C: NUR2635, McGill University, winter semester, 2010), and updated for the necessity of the present Thesis.

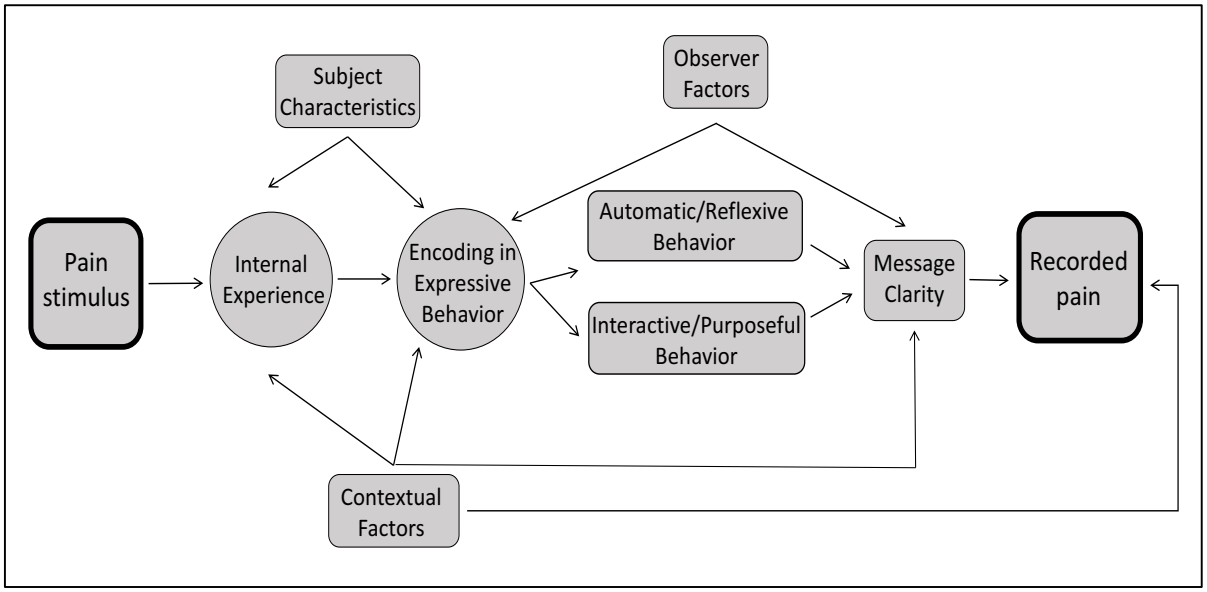
1.2.1 The problem of pain recognition and measurement

There have been substantial advances in the recognition, prevention and treatment of companion animal pain, particularly within the last two to three decades; for instance, the World Small Animal Veterinary Association has established a Global Pain Council to promote recognition of pain and to minimize its prevalence and impact (Mathews, Kronen et al. 2014). However, improved animal pain management is largely due to enhanced recognition of the importance of preemptive analgesia for surgical procedures and analgesic intervention for medical and traumatic conditions, both based primarily on the expected level of pain associated with the disease or procedure (Dohoo and Dohoo 1996, Epstein, Rodan et al. 2015, Mathews, Kronen et al. 2014). The problem of pain identification, and therefore of successful management, persists (Mathews, Kronen et al. 2014, Merola and Mills 2016, Taylor and Robertson 2004). One remedy proposed for the difficulty of detecting animal pain is evaluation of response to treatment to measure the presence and severity of pain (Mathews, Kronen et al. 2014). However, signs of animal pain are sometimes overlooked or mistaken for other problems, and this under-recognition (Epstein, Rodan et al. 2015, Taylor and Robertson 2004), along with practitioner fears of adverse secondary effects related to analgesic medications (Sparkes, Heiene et al. 2010, Taylor and Robertson 2004), may result in under-treatment of pain. This is important because unalleviated pain negatively impacts welfare (Merola and Mills 2016).

The experience and expression of pain vary between individuals, even in the presence of an identical degree of tissue damage, and are influenced by factors such as pain processing, behavioral and experiential differences, developmental stage, and contextual (including observer) factors (Fernandez and Turk 1992, Hadjistavropoulos and Craig 2002, Williamson

and Hoggart 2005). In human medicine, the gold standard for pain assessment is arguably patient self-report (McCaffery 1968), which is impossible in animals. The implication is the immediate injection of a source of error into pain evaluation in veterinary medicine: the observer (proxy) who must interpret the level of pain in order to proceed with decisions regarding analgesic intervention (Merola and Mills 2016). Hence, error in the evaluation can come from differences in patient responses to pain, and differences in observer training, observational capacities, preconceptions, and other factors (Hadjistavropoulos and Craig 2002). Pain has interdependent sensory and affective/emotional aspects (Fernandez and Turk 1992), and different behavioral manifestations of pain may also reflect different aspects of the pain experience (Merola and Mills 2016). A recent review of behavioral measures of pain in cats found that acute measures, including pain scales, tended to focus on the sensory domain of pain, while tools for assessing chronic pain conditions such as OA and degenerative joint disease (DJD) tended to employ evaluations of the affective domain of pain (Merola and Mills 2016). See Figure 1.2.1 for a diagrammatic representation of various influences on the communication of animal pain.

Figure 1.2.1: Factors influencing the communication of animal pain.



(Adapted from Hadjistavropoulos and Craig, 2002)

1.2.1.1 Methods of pain assessment in animals

In clinical veterinary medicine, pain is typically evaluated using a combination of objective and subjective assessments. The objective methods employed in practice consist primarily of physiologic measures (*e.g.*, heart rate, respiratory rate, body temperature, blood pressure, appetite/food intake, weight gain/loss) (Anil, Anil et al. 2002, Weary, Niel et al. 2006). Unfortunately, these physiologic parameters are not specific to pain, being susceptible to influence by other factors, such as non-painful illness, exercise or stress (Anil, Anil et al. 2002, Duncan 2005, Molony and Kent 1997). Additional methods used for evaluating animal pain in veterinary medical research include measurements of: locomotor activity (using collar-mounted devices in cats and dogs, *e.g.*, (Lascelles, Hansen et al. 2007)), ground reaction forces (kinetics, *e.g.*, (Schnabl and Bockstahler 2015)), angular joint movements (kinematics, *e.g.*, (Guillot, Gravel et al. 2015)), quantitative sensory testing (using thermal or mechanical stimuli, *e.g.*, (Guillot, Moreau et al. 2013, Knazovicky, Helgeson et al. 2016)), body surface temperature patterns (thermography, *e.g.*, (Vainionpää, Raekallio et al. 2013)), biomarkers (such as catecholamines and cortisol, *e.g.*, (Smith, Allen et al. 1996)), and brain activity (functional neuroimaging, *e.g.*, (Guillot, Chartrand et al. 2015)).

Subjective pain evaluation tends to depend on owner reports and/or interpretation of behavior (*e.g.*, changes in normal/usual behaviors or the appearance of new behaviors), as well as on the observations of veterinarians/veterinary staff members (*e.g.*, based on behavior, posture, and gait, either in the absence of any interference, or in association with general interactions or with palpation and manipulation of body parts). These methods rely heavily on human observation and interpretative abilities, requiring familiarity with species-specific behaviors, and benefiting from prior knowledge of the individual's usual behavior. Pain scales are standardized subjective assessment tools that aim to reduce error in subjective evaluations; an example of a type of pain scale in common use in clinical veterinary practice is a lameness scale (*e.g.*, for grading lameness in horses or dairy cattle) (Weary, Niel et al. 2006). Additional discussion of objective and subjective measures of animal pain will be presented in Sections 1.3 and 1.4.

1.2.2 Types of pain scales

It has been recommended that dogs and cats be examined for pain at every veterinary contact (Epstein, Rodan et al. 2015, Mathews, Kronen et al. 2014). The American Animal Hospital Association (AAHA)/American Association of Feline Practitioners (AAFP) Pain Management Guidelines for Dogs and Cats Task Force strongly advises the use of behavioral assessment, and specifically advocates standardized pain scoring tools, "pain scales", to minimize the subjectivity of interpretation and hence the influence of observer-associated bias on pain evaluation (Epstein, Rodan et al. 2015). The following section describes several common types of pain scales.

1.2.2.1 Single-item scales

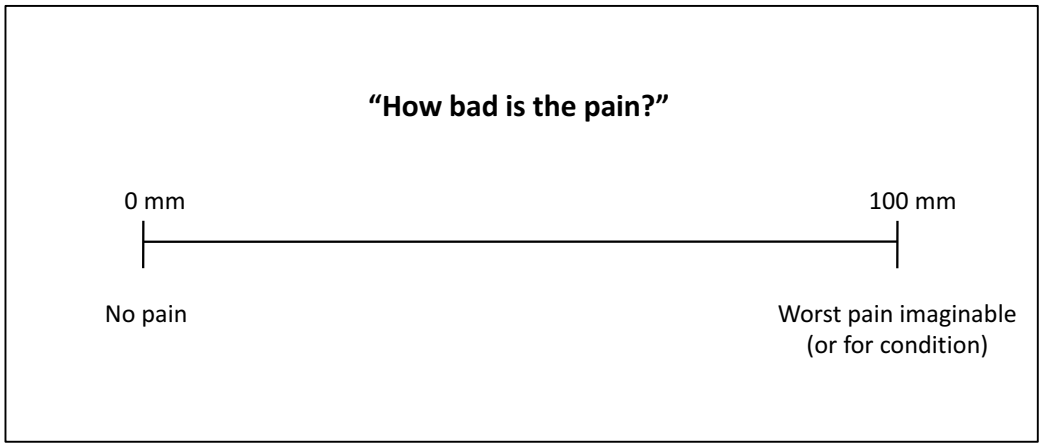
Single-item rating scales contain only a single question or statement, which may be used to assign a global pain rating (*e.g.*, "How bad is the pain?"); consequently, these scales are unidimensional (Martinez-Martin 2010, von Baeyer and Spagrud 2007). They are simple and quick to use (Hartrick, Kovan et al. 2003, Kahl and Cleland 2005). However, they are highly subjective, and when used by a proxy/observer rather than for self-report, they are particularly susceptible to observer bias (von Baeyer and Spagrud 2007). Lack of standardization (*e.g.*, differences in terms used to describe scale categories or extremes) and ambiguity can lead to poor reliability, and if the number of possible scores is low, these scales may be inadequately sensitive/responsive (Martinez-Martin 2010).

1.2.2.1.i Visual analogue scale

The visual analogue scale (VAS) typically consists of a 10-cm horizontal line with markers at each end to indicate the lowest and highest possible responses, *e.g.*, for a simple pain assessment, "no pain", and "worst pain imaginable" (Williamson and Hoggart 2005). See Figure 1.2.2 for an example of a VAS. The observer places a mark across the line at the point between the two extremes that they feel best represents the subject's level of pain, and the score assessed on this basis is the measured distance from the "0", or lowest end, to the mark. It yields continuous, but not necessarily linear, data. That is, the physical distance between

points on the scale does not necessarily represent consistent amounts of change in pain (*i.e.*, the difference between 2.5 and 3.5 may not be equivalent to that between 7.5 and 8.5, and a score of 5 does not mean twice as much pain as a score of 2.5), meaning that score comparisons (between or within individuals) must be interpreted with caution (Kersten, White et al. 2014).

Figure 1.2.2: Example of a visual analog scale.



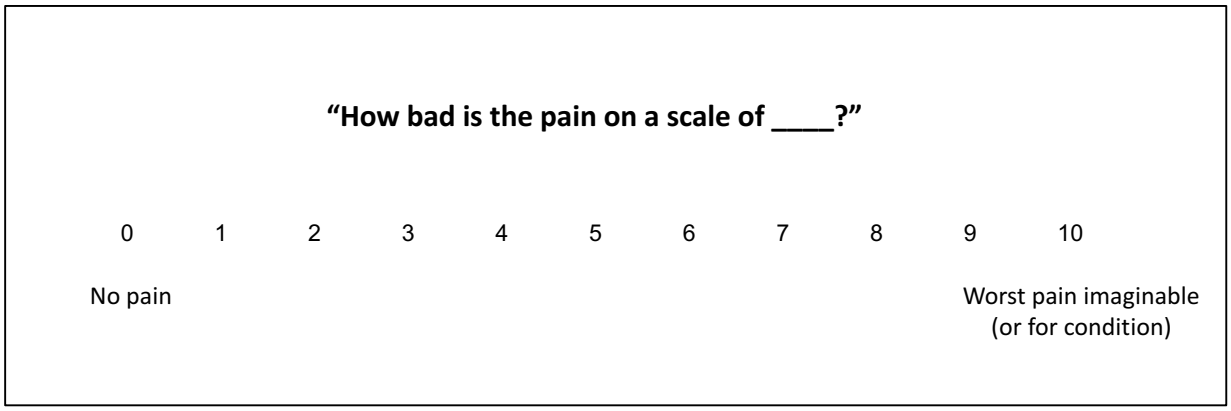
A major disadvantage of this type of scale in veterinary medicine is that it relies completely on the observer's ability to identify and interpret signs of pain in the patient, making it susceptible *e.g.*, to user inexperience (Benito-de-la-Víbora, Lascelles et al. 2008). In addition, sensitivity may be affected by the range of the scale, as users may cluster scores at the low end even in painful animals, if "worst possible pain" is used as an anchor; modification of the right-hand (upper limit) anchor of the VAS based on the intended use (*e.g.*, "worst possible pain for (the particular condition/procedure))" may reduce this (Benito-de-la-Víbora, Lascelles et al. 2008, Lascelles, Cripps et al. 1995). The "dynamic and interactive" VAS (DIVAS) and "interactive" VAS (IVAS) use a standardized assessment procedure; this type of scale has been recommended for use in post-operative pain (Epstein, Rodan et al. 2015). The DIVAS score is based upon all of the following: 1) observation of the patient without interaction (*e.g.*, from outside the cat's cage), 2) observation of the patient during interaction (*e.g.*, with the cat's cage open, and while encouraging the cat to move about), and 3) observation of the patient's response to palpation of the painful region (*e.g.*, surgical site)

(Epstein, Rodan et al. 2015, Lascelles, Cripps et al. 1995). The IVAS and DIVAS may have better acute post-operative pain detection than the VAS alone, but this has not been definitively confirmed (Benito-de-la-Víbora, Lascelles et al. 2008, Cambridge, Tobias et al. 2000, Lascelles, Cripps et al. 1995). Additionally, reported evaluation procedures for the DIVAS/IVAS assessment vary (Cambridge, Tobias et al. 2000, Lascelles, Cripps et al. 1995).

1.2.2.1.ii Numerical rating scale

The numerical rating scale (NRS) is a scale that does not provide defined categories of pain, but rather asks the rater to assign a whole number between two extreme values (variable scale, *e.g.*, 0 to 10, 1 to 10, or 1 to 5), that represent "no pain" and "worst possible pain" (Williamson and Hoggart 2005). See Figure 1.2.3 for an example of an NRS. Like the other single-item scales described here, the intervals between numbers cannot be assumed to be equal (*i.e.*, although the scale implies interval level measurement, the scores obtained are probably more accurately considered ordinal level) (Hartrick, Kovan et al. 2003). The VAS and NRS may yield different results and are therefore not interchangeable, even given the same apparent scale (*i.e.*, 0 to 10). In humans, the NRS has been suggested not to be as sensitive to small changes in level of pain as is the VAS; and it can yield (sometimes systematically) different scores (Hartrick, Kovan et al. 2003, Holdgate, Asha et al. 2003, Kahl and Cleland 2005, Williamson and Hoggart 2005). One feline study reported ability of an NRS to detect analgesia following ovariohysterectomy, when a VAS did not; however, it was noted that neither scale had been validated for the study use, and that inexperience of the rater may have influenced the results (Benito-de-la-Víbora, Lascelles et al. 2008).

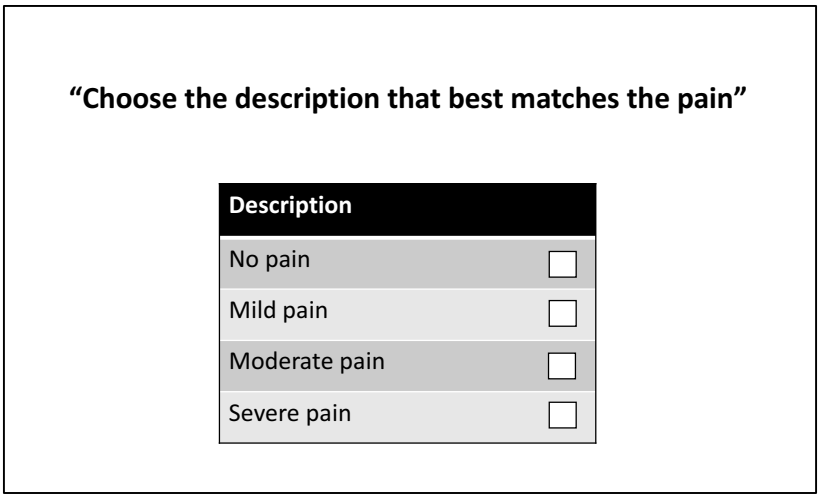
Figure 1.2.3: Example of a numerical rating scale.



1.2.2.1.iii. Simple descriptive scale

The simple descriptive scale (SDS), also called a verbal rating scale (VRS), is an ordinal-level scale consisting of defined categories, usually four to six, ranging from minimum to maximum, *e.g.*, "no pain", "mild pain", "moderate pain", "severe pain" (Lascelles, Henderson et al. 2001, Williamson and Hoggart 2005). See Figure 1.2.4 for an example of an SDS. The common practice of assigning numerical scores to the categories (*e.g.*, 0, 1, 2, 3) does not signify that the intervals between categories are equal (Williamson and Hoggart 2005). This scale is simple to use and definitions of categories provide some guidance to the user. However, collapsing the degree of pain into a small number of categories makes it less sensitive than the VAS and some versions of the NRS, meaning that the scale may not be able to identify small yet clinically important differences in pain state (*e.g.*, in response to therapy or when analgesia is wearing off) (Williamson and Hoggart 2005). One feline study reported distinction of surgically onychectomized/tenectomized from control (anesthesia alone) groups, using a four-response category SDS for palpation of the forelimbs at the time of peak pain, post-operatively (Cambridge, Tobias et al. 2000). The feline medical literature contains various examples of the use of these types of scales to assess such pain-related characteristics as appetite, lameness, and general demeanor (*e.g.*, (Clarke and Bennett 2006, Lascelles, Henderson et al. 2001)).

Figure 1.2.4: Example of a simple descriptive scale.



1.2.2.2 Multi-item scales

Scales with multiple items are referred to as composite scales; these are made up of a number of questions or statements evaluating either one (unidimensional) or several (multidimensional) dimensions associated with the construct of interest (Martinez-Martin 2010). This design allows for a more detailed evaluation than does a single-item scale, thereby potentially improving reliability and sensitivity/responsiveness, particularly for complex constructs; however, they tend to be more complicated and time-consuming to use than are single-item scales, both because of the time to complete the scale, and the need for score calculation (Martinez-Martin 2010). Generally, it is recommended that the components of these scales should be related, so that the total score has meaning (Martinez-Martin 2010). The single-item scales described above each can be used as a part of a composite pain scale, for instance, to obtain measurements of different aspects of pain, or behaviors considered to represent either pain or the absence of it (*e.g.*, attention to surgical site, activity, attitude, *etc*.). Examples of multi-item scales validated or partially validated for use in cats include: the Composite Measures Pain Scale – Feline (CMPS-F) (Calvo, Holden et al. 2014, Holden, Calvo et al. 2014, Reid, Scott et al. 2017), the Universidad Estadual Paulista (São Paulo State University)-Botucatu Multidimensional Composite Pain Scale (UNESP-Botucatu MCPS) (Brondani, Luna et al. 2011, Brondani, Luna et al. 2012, Brondani, Luna et al. 2013), the 4A-

Vet (Gauthier, Holopherne-Doran et al. 2015, Laboissière 2006), the Client-Specific Outcome Measures scale (CSOM) (Gruen, Griffith et al. 2015, Lascelles, Hansen et al. 2007), an unnamed feline musculoskeletal pain scale reported by Bennett and Morton (Bennett and Morton 2009), and the Feline Musculoskeletal Pain Index (FMPI) (Benito, Depuy et al. 2013, Benito, Hansen et al. 2013, Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Zamprogno, Hansen et al. 2010). Table 1.2.1 provides a brief overview of these scales.

Table 1.2.1: Examples of composite pain scales with reported evidence of validity for feline pain assessment.

Scale	Type of Pain	Context and Observer	Scale Evaluation Procedure
CMPS-Feline	Acute (various causes)	Hospital Veterinarian/veterinary staff	Observation of cat in cage Incorporates pain face scale
UNESP-Botucatu MCPS	Acute postoperative	Hospital Veterinarian/veterinary staff	Observation Interaction/handling Physiologic (BP, appetite)
4A-Vet	Acute postoperative	Hospital Veterinarian/veterinary staff	Observation Interaction/handling Physiologic assessment (HR)
CSOM	Chronic DJD	Home Owner	Owner recall (specific activities selected by owner)
Feline musculo-skeletal pain scale	Chronic musculo-skeletal	Home Owner	Owner recall
FMPI	Chronic DJD	Home Owner	Owner recall

Legend: CMPS = Composite Measures Pain Scale; UNESP-Botucatu MCPS = UNESP-Botucatu Multidimensional Composite Pain Scale; BP = arterial blood pressure; HR = heart rate; CSOM = Client-Specific Outcome Measures; DJD = degenerative joint disease; FMPI = Feline Musculoskeletal Pain Index.

1.2.2.2.i Inclusion of physiologic assessments in multi-item pain scales

Some multi-item scales include physiologic as well as behavioral assessments (*e.g.*, the 4A-Vet scale and the UNESP-Botucatu MCPS). Such assessments may include: heart rate, respiratory rate, blood pressure, body temperature, and appetite; either absolute values or changes from baseline (or percent changes) may be considered. It is generally accepted that behavioral observation for detection of pain is more accurate than is the use of physiologic measures (Epstein, Rodan et al. 2015), but physiologic measures may augment behavioral assessments. One study evaluating physiologic assessments for detection of pain post-ovariohysterectomy in cats found that only systolic blood pressure detected post-operative stress and pain (based on increased cortisol); heart rate, respiratory rate, and rectal temperature did not (Smith, Allen et al. 1996).

1.2.2.2.ii Pain face scales

Scales may also assess components of a "pain face", *i.e.*, a facial expression consistent with pain. Such scales have been reported for laboratory, farm, and companion animals, and include assessment of different components of the facial expression, separately (Dalla Costa, Minero et al. 2014, Descovich, Wathan et al. 2017, Guesgen, Beausoleil et al. 2016, Holden, Calvo et al. 2014, Keating, Thomas et al. 2012, Langford, Bailey et al. 2010, Leach, Klaus et al. 2012, McLennan, Rebelo et al. 2016). Hence, they fall under the category of multi-item scales. They may be used by themselves, or incorporated into other scales, *e.g.*, as is done in the University of Glasgow's CPMS for acute feline pain (Reid, Scott et al. 2017).

1.2.2.2.iii Personalized multi-item scales

Although most multi-item scales are based on standardized items, one that is not is the Client-Specific Outcomes Measures (CSOM), which has been described for use in monitoring feline DJD pain (Figure 1.2.5) (Gruen, Griffith et al. 2015, Lascelles, Hansen et al. 2007, Lascelles, DePuy et al. 2010). This scale involves owner interview to select activities (items) affected by DJD in the particular feline patient (Lascelles, Hansen et al. 2007).

Figure 1.2.5: Client-Specific Outcome Measures scale.

CLIENT SPECIFIC OUTCOME MEASURES—ACTIVITY*

Indicate how problematic these activities are compared to when your cat was normal, or did not have osteoarthritis. Comparison is to when he/she was _____ years old.

Problems in mobility related to osteoarthritis in your cat	*No problem*	*A little problematic*	*Quite problematic*	*Severely problematic*	*Impossible*
1.					
2.					
3.					
4.					
5.					

(Reprinted from the Journal of Veterinary Internal Medicine, 21(3), Lascelles, B.D. *et al.* Evaluation of client-specific outcome measures and activity monitoring to measure pain relief in cats with osteoarthritis. Pages 410-416 (2007), with permission from Elsevier.)

1.2.3 Pain scale development

1.2.3.1 Items

Scale items are typically generated *via* some combination of: 1) literature review, 2) collection of new research data, 3) expert opinion, 4) clinical observation, 5) focus groups, and 6) interviews with targeted respondents (Duhn and Medves 2004, Streiner and Norman 2008). For example, items for the FMPI were generated *via* focus groups made up of cat owners (Zamprogno, Hansen et al. 2010). An attempt is made to identify all potentially relevant items at this stage (Duhn and Medves 2004). Subsequently, items may be reworded or removed based on an evaluation of interpretability, which includes aspects such as reading level and item length, and the presence of ambiguity or jargon (Streiner and Norman 2008). Further item selection is based on aspects of the validation process, described below.

1.2.3.2 Response options

Item scaling, or the selection of response options, involves determining whether responses will be dichotomous (*e.g.*, present/absent), ordinal, or even continuous (*e.g.*, items may be scored on a VAS) (Duhn and Medves 2004). Examples of ordinal response options include unipolar adjectival (ranging from all to none; *e.g.*, excellent, good, fair, poor), and bipolar Likert scales (*e.g.*, strongly agree, agree, neither agree nor disagree, disagree, strongly disagree) (Streiner and Norman 2008). Scales will yield different types of data based in part on this selection; evaluation is required to determine what descriptive and inferential statistics are appropriate (Martinez-Martin 2010). Intervals between response options are not necessarily equal (as discussed above, regarding single-item scales), although various methods exist to approximate interval-level scaling (*e.g.*, as has been described for the Composite Measures Pain Scale for dogs (Morton, Reid et al. 2005)).

1.2.3.3 Combining items into a scale

When combining items to generate a total scale score, it must be determined: 1) whether individual items should be weighted based on their importance, 2) how to handle missing items, 3) how the total score will be presented, and 4) what will be the cut-off thresholds for classification *e.g.*, as painful/non-painful, and for inferring a change in status, *e.g.*, worsening/improved pain (Streiner and Norman 2008). The value of weighting scores is unclear; it may be helpful in scales containing few (*e.g.*, fewer than 40) items, particularly if items are not homogeneous (Streiner and Norman 2008). Two ways of addressing missing items are to base the scale score calculation only on completed items (which is likely not to distort outcomes if < 5% of items are missing), or to assign a score of zero to missing items (which may underestimate the subject's true score) (Streiner and Norman 2008). The total score may be a sum of the item scores, or may be transformed in some way (*e.g.*, into percentiles, *z*-scores, or *T*-scores) in order to facilitate between-scale comparisons, *e.g.*, for research evaluating the performance of multiple scales in a specific context (Streiner and Norman 2008). An example of a simple transformation is presentation of the total scale score as the percentage of the possible total score, as was reported for the FMPI (Gruen, Thomson et al. 2016). Methods of establishing cut-off thresholds can be applied to diagnosis (*e.g.*, use of

receiver operating characteristic curves, for instance, to set score thresholds for OA *vs.* non-OA cats), and are also involved in detection of treatment efficacy, *e.g.*, as the minimum clinically important difference or the minimally important difference/change (MCID or MID/MIC; the smallest change in score considered important by a human patient) (Copay, Subach et al. 2007, de Vet, Terwee et al. 2006).

1.2.3.4 Translation

Translation is sometimes needed if a scale is to be used in a new geographical population (*e.g.*, a different country). This requires confirmation that the scale items are conceptually equivalent when translated; one aspect of this is back-translation and comparison of the original and twice-translated scale for discrepancies (Streiner and Norman 2008).

1.2.4 Pain scale validation

The process of pain scale validation is complex. The literature describing it can be confusing, in part due to differences in the terminology used. The following section describes common aspects of validation.

1.2.4.1 A note on classic vs. contemporary validation theory

It should be noted that perspectives on rating scale validation have shifted since the concept began to be developed in the mid-20th century (Gélinas, Loiselle et al. 2008). The classic approach to validation seeks to determine how well a scale measures the construct of interest, by considering a so-called "trinity": content, criterion, and construct validity, while the contemporary approach is defined as "the degree to which evidence and theory support the interpretations of test scores entailed by proposed uses of tests" (American Educational Research Association (AERA), American Psychological Association (APA) et al. 1999, Goodwin 2002). A detailed discussion of the evolution of rating scale validation is beyond the scope of this review; suffice it to say that: 1) the contemporary approach emphasizes that validity is not a characteristic of a scale itself, rather, evidence may support the validity of scale use for a specific purpose (*i.e.*, in particular types of subject, context, *etc.*), 2) the

contemporary method advocates a unitary approach based on a body of supportive evidence, rather than a categorical approach to validation, 3) there is substantial overlap between aspects of the classic and contemporary approaches to validation, and 4) one added aspect of validation in the contemporary approach is the consideration of evidence based on the consequences of testing (Gélinas, Loiselle et al. 2008, Goodwin 2002). Studies vary with respect to their adherence to the contemporary *vs.* the classic approach, and differences in terminology relating to scale validation concepts can lead to confusion (*e.g.*, construct validity is sometimes considered to encompass all aspects of validation) (Gélinas, Loiselle et al. 2008). The following discussion describes the types of evidence pertinent to scale validation, as well as how they fit into each of the classic and contemporary views, giving examples of related terminology.

1.2.4.2 Aspects of validity

1.2.4.2.i Evidence based on test content

This type of evidence of validity, called content validity in the classic view, considers the extent to which items comprehensively represent the underlying construct (*e.g.*, feline OA pain) (Gélinas, Loiselle et al. 2008). Validation of this type typically takes the form of expert evaluation to determine how well the scale and its subparts and items match the construct and the test purpose, including assessments of the clarity, relevance, and importance of items, and to what degree the scale may be under-representative of the construct, or conversely, may contain irrelevant components (Goodwin 2002).

1.2.4.2.ii Evidence based on response processes

This type of evidence, which forms part of construct validity in the classic approach, evaluates the extent to which scale responses fit the construct of interest, and are specific to it (Gélinas, Loiselle et al. 2008). Evidence of this type may be obtained *via* analysis of respondent interviews, comparison of answers given by different subgroups of subjects, and evaluation of how persons administering the scale collect, record, and interpret data (Goodwin 2002).

1.2.4.2.iii Evidence based on internal structure

This type of evidence, which also forms part of construct validity in the classic view of validation, refers to the degree to which relationships between test items and dimensions are representative of the construct of interest (Cook and Beckman 2006, Gélinas, Loiselle et al. 2008). Examination of the internal structure of a scale may take the form of item analyses to evaluate inter-item relationships (*e.g.*, evaluations made to assess internal consistency; see below), factor analysis, and differential item functioning studies (Cook and Beckman 2006, Goodwin 2002). Examples of veterinary pain scales for which factor analysis has been described include the Canine Brief Pain Inventory (Brown, Boston et al. 2007) and the University of Glasgow Health-Related Quality of Life Scale for dogs (Wiseman-Orr, Scott et al. 2006).

1.2.4.2.iv Evidence based on relations to other variables

This type of evidence refers to the nature and extent of relationships of scale outcomes with related and unrelated outcomes, and hence incorporates aspects of classic criterion and construct validity (Gélinas, Loiselle et al. 2008). For instance, it includes comparisons of scale outcomes for distinct subgroups (*e.g.*, cats with and without OA), and evaluation of responsiveness of scales to intervention (*e.g.*, analgesic treatment) (Goodwin 2002). Criterion validity refers to the degree to which the scale outcomes correspond with a gold standard, if one exists. It may be assessed as concurrent (*i.e.*, scale and gold standard test undertaken within the same time frame) or predictive (*i.e.*, scale outcome prediction of future gold standard outcomes) (Gélinas, Loiselle et al. 2008). The facets of construct validation that consider relationships with other variables are convergent (related) and discriminant or divergent (unrelated) (Gélinas, Loiselle et al. 2008). Pain is an attribute that is somewhat abstract, *i.e.*, a "construct" or a "latent variable"; it lacks a unit of measurement and is not directly observable, so that its measurement is always indirect (Martinez-Martin 2010). It has been stated that there is no gold standard for assessing pain in cats (Epstein, Rodan et al. 2015). Hence, it could be argued that criterion validation cannot be performed for pain scales, and that only convergent (construct validation) can be performed (Gélinas, Loiselle et al. 2008).

1.2.4.2.v Evidence based on consequences of testing

This type of evidence relates to the extent to which use of the rating scale yields benefits (anticipated or unanticipated) or negative consequences, and how consequences vary for different groups of subjects (Gélinas, Loiselle et al. 2008, Goodwin 2002). For example, if a group of cats were screened for OA using a pain scale, some would be classified as OA, and others as non-OA, based on the scale outcome; a positive consequence of testing would be identification and treatment of cats with OA pain. However, negative consequences could include inappropriate treatment or unnecessary follow-up testing due to misclassification of non-OA cats as OA. This type of validity evidence is somewhat controversial with respect to how and whether it should be implemented (Gélinas, Loiselle et al. 2008, Goodwin 2002).

1.2.4.3 Reliability

In addition to validation, psychometric testing of rating scales requires verification of reliability (Gélinas, Loiselle et al. 2008). Reliability essentially refers to the tool's relative freedom from random and systematic error associated with measurement (Streiner and Norman 2008), and generally is broken down into three categories: 1) inter-rater reliability, intra-rater reliability, and internal consistency reliability (Crellin, Sullivan et al. 2007).

1.2.4.3.i Inter- and intra-rater reliability

Classical test theory states that the observed score with a measurement tool is equal to the sum of the true score and measurement error; reliability coefficients attempt to estimate the proportion of the observed score variance that results from true score variance, *vs.* that due to error (Hallgren 2012), according to the following formula (Streiner and Norman 2008):

$$Reliability = \frac{Subject\ Variability}{Subject\ Variability + Measurement\ Error}$$

Reliability coefficients are therefore expressed on a standardized scale of zero to one, with zero indicating that all true score variability is due to measurement error (*i.e.*, no reliability), and with one indicating that there is no measurement error (*i.e.*, perfect reliability)

(Koo and Li 2016, Streiner and Norman 2008). Because it is dependent on the variability between subjects, reliability must be considered, not as an intrinsic quality of a rating scale, but as a measure of how the rating scale performs in a given population and context (Streiner and Norman 2008). Reliability testing must therefore be performed under conditions similar to those in which the scale is targeted for use (Streiner and Norman 2008).

1.2.4.3.i.a Inter-rater reliability

This type of reliability refers to the extent of agreement between different raters using the scale independently to assess the same subject, at the same time. It is particularly important in the context of observational scales (*i.e.*, those completed by a proxy) (Gélinas, Loiselle et al. 2008). Inter-rater reliability therefore reflects the degree of measurement error in the scale, that is associated with differences in scoring between users/coders (Hallgren 2012). Reliability coefficients reported to assess this type of reliability are intra-class correlation coefficients (ICCs; based on analysis of variance), kappa coefficients for binary (*e.g.*, Cohen's kappa (Cohen 1960)) and weighted kappa coefficients (Cohen 1968) for more than two outcomes (based on percentage agreement corrected for chance), and Pearson or Spearman correlations (based on how well the relationship between two sets of scores approximates a straight line, for continuous and ordinal data, respectively) (Gélinas, Loiselle et al. 2008), as well as Bland-Altman plots (based on the relationship of the differences in the pairs of ratings to their means) (Altman and Bland 1983), or paired t-tests (or nonparametric alternatives) (Koo and Li 2016). Percentages of agreement are also sometimes used, but not recommended as they do not correct for agreement that arises due to chance and therefore overestimate reliability (Koo and Li 2016). Generally, Kappa coefficients and ICCs are recommended (Koo and Li 2016, Streiner and Norman 2008). Some authors advise the use of ICCs for any but the most simple of two x two tables; this is in part because ICCs accommodate more than two raters, while weighted Kappa coefficients cannot (Streiner and Norman 2008). Criticisms of the other methods include: 1) that the Pearson and Spearman correlations do not assess agreement, merely consistency, and may therefore not detect systematic differences between raters (Giavarina 2015), and 2) that the Bland-Altman method and paired t-tests assess agreement but neglect correlation (Koo and Li 2016). It has also been

argued that the Bland-Altman method does not provide any information beyond that obtained via ICC analysis (Streiner and Norman 2008).

Recommendations for minimum reliability coefficients vary in part based on whether the scale is intended for use to make decisions regarding individual patients (requiring higher reliability) or whether it is to be used for research; a minimum of 0.75 for the scale total has been proposed (Streiner and Norman 2008). Individual scale item inter-rater reliability coefficients may vary somewhat, but items with low values can be expected to add to overall scale error (*i.e.*, to worsen its reliability). An example of guidelines for interpretation of ICC coefficients is: < 0.40 = poor, 0.40-0.59 = fair, 0.60-0.74 = good, 0.75-1.00 = excellent (Cicchetti and Sparrow 1981). For Kappa coefficients, commonly used guidelines are as follows: < 0.00 = poor, 0.00-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial, 0.81-1.00 = almost perfect (Landis and Koch 1977). Low reliability in a sample more homogeneous than the target population may not be problematic, because increased heterogeneity in the target group would increase reliability coefficients (Koo and Li 2016, Streiner and Norman 2008).

1.2.4.3.i.b Intra-rater reliability

This type of reliability refers to the extent of agreement between ratings by the same user, over time; it is also referred to as test-retest reliability, or stability (Gélinas, Loiselle et al. 2008). Ratings must be performed far enough apart to make it unlikely that evaluators remember their previous scoring, and close enough in time that the construct being measured has not changed; a two- to 14-day interval has been recommended (Streiner and Norman 2008). One way to ensure no change in the underlying construct is to use a videotaped subject (*i.e.*, precisely the same scenario) for the repeated scale assessments. Poor intra-rater reliability may indicate either that the measuring instrument is not stable when used to assess the same subject over time, that the construct being measured has itself changed, or that the rater has been sensitized by the first administration of the test (Hallgren 2012, Streiner and Norman 2008). Coefficients used to assess intra-rater reliability are the same as those used to evaluated inter-rater reliability. Generally, results are expected to be higher for the former than for the

latter, as there is no error contributed by differences between raters themselves (Streiner and Norman 2008).

1.2.4.3.ii Internal consistency reliability

This type of reliability refers to the extent to any one item on a scale is a good indicator of the performance of any other item on the same scale (Gélinas, Loiselle et al. 2008); in other words, it describes scale homogeneity (Streiner and Norman 2008). Moderate correlations between items and between each item and the scale total support that all items are measuring the same construct (Streiner and Norman 2008). Measurement error reflected *via* poor internal consistency may arise due to imprecision, inaccuracy, or poor scaling of items (Hallgren 2012). Some authors advise that evaluation of internal consistency is questionable in scales with few items or with only one dimension (Gélinas, Loiselle et al. 2008), while others suggest that multidimensional scales should have subscales assessed independently for internal consistency (Streiner and Norman 2008). There are also cases in which internal consistency is not considered essential; some measures emphasize ability to discriminate groups over the need for all items to assess one particular trait (Streiner and Norman 2008).

The two statistics commonly used to report internal consistency are Kuder-Richardson (KR-20) for dichotomous items, and Cronbach's alpha for items with more response options (Gélinas, Loiselle et al. 2008). Cronbach's alpha is an extension of KR-20 and produces the same results if used for dichotomous items (Streiner and Norman 2008). These statistics yield reliability coefficients between zero and one, with values close to zero indicating a low degree and values close to one indicating a high degree of homogeneity; a result between 0.70 and 0.90 has been recommended as indicating sufficient interrelatedness of items, without redundancy. However, increasing the number of items on the scale increases the coefficient (Streiner and Norman 2008). Cronbach's alpha may occasionally produce negative reliability coefficients (instead of a coefficient between zero and one, as described above); this indicates a problem in the scoring or construction of the scale (Streiner and Norman 2008). Other methods of assessing internal consistency include: item-total and inter-item correlations, and split-half correlations (in which scale items are randomly divided into two subscales and these two are correlated) (Streiner and Norman 2008). The first may be somewhat unwieldy in

scales with a large number of items (having to be repeated for each item), and the second, because it artificially shortens the scale by splitting it, underestimates internal consistency. Split-half correlations can also yield variable results depending on what items are included in each half (Streiner and Norman 2008).

1.2.4.4 Aspects of the validation process based on end-user perceptions

1.2.4.4.i Face validity

Face validity is typically defined as whether the scale appears to measure what it is meant to measure (Streiner and Norman 2008). This assessment is made, not by experts, but by respondents, and therefore reflects an aspect of acceptability of the tool to the end-users (Streiner and Norman 2008). Evaluation of face validity can be performed by asking similar questions of end-users to those asked of experts in the context of content validation, *i.e.*, regarding clarity and importance of items, and relevance of response options.

1.2.4.4.ii Comprehensibility

Scale comprehension is related to face validity and to acceptability of the instrument. Evaluation of comprehensibility can include assessments of readability, *i.e*, the ease with which a reader can understand the text, and cognitive interviews with respondents. Various indices of readability exist, and are typically based on factors such as sentence length and complexity (including word difficulty) (Klare 1974). Readability indices are frequently presented as the minimum school grade level necessary to understand the text. Cognitive interviews permit verification that the scale is understood by respondents in the manner which was intended by developers. This is accomplished by evaluating respondent thought processes in response to the scale (*e.g.*, by having them think aloud, or through the use of verbal probing), and hence contributes to evidence of validity based on response processes (Patrick, Burke et al. 2011).

1.2.4.4.iii Feasibility and clinical utility

Additional important aspects of a scale, if it is to be used clinically, are its feasibility, *i.e.*, its ease of use, and its clinical utility, *i.e.*, its usefulness or how informative it is, in the clinical setting (Duhn and Medves 2004). Criteria used to assess feasibility may include the length of time needed for training to use the scale, the time needed to conduct the scale evaluation, the time and method used to complete scoring, the clarity of scale instructions, and the scale structure and perceived complexity (Gélinas 2010). Criteria used to assess clinical utility may include whether the evaluator would recommend the scale's use, and its perceived helpfulness in practice, including its impact on (*e.g.*, pain) assessments (Gélinas 2010).

1.2.5 How to use pain scales

Validation studies give the scale user an understanding of how the scale behaves in a given context and population. It is incorrect, however, to refer to a scale as generally "valid", as validity relates specifically to the use for which the scale has been evaluated. A scale validated for evaluating post-ovariohysterectomy pain in cats (*e.g.*, the UNESP-Botucatu MCPS) may therefore perform differently if used to evaluate cats with other types of visceral or orthopedic pain, for instance. A recent review lamented the lack of feline pain scales that apply across a broad range of conditions, and noted that existing scales had been validated in relatively limited contexts (Merola and Mills 2016). It has been recommended that pain scales should be used as an adjunct to preemptive pain assessments (*i.e.*, anticipated severity of pain based on procedure or condition), as well as subjective assessments of pain and physical examination findings in a given animal, to increase pain detection (Epstein, Rodan et al. 2015).

1.3 Feline Osteoarthritis

1.3.1 What is osteoarthritis?

1.3.1.1 Disease overview

Osteoarthritis is a degenerative disease that causes progressive pain and loss of function (Bennett, Zainal Ariffin et al. 2012, Kerwin 2010) It is common in humans and in many animal species, but diagnosis in cats has historically been infrequent (Bennett, Zainal Ariffin et al. 2012), despite the species' popularity as a pet. Studies over the past two decades have demonstrated that there is a high prevalence of structural and radiographic changes consistent with the disease in this species, and that this prevalence increases with age (Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011). Further study has determined that cats show subjective and objective improvements in response to analgesic treatment (Bennett and Morton 2009, Clarke and Bennett 2006, Gruen, Griffith et al. 2014, Gruen, Thomson et al. 2016, Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2007, Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017). It therefore appears that OA is prevalent in cats, that it is a clinical disease causing pain and affecting mobility just as it does in other species, and that the likely cause of its historically low rate of diagnosis is the subtlety of its signs in the cat, or misinterpretation of these signs as simply being due to nonspecific aging (Bennett and Morton 2009).

1.3.1.2 Terminology: osteoarthritis vs. degenerative joint disease

Some difficulty is encountered in assimilating the findings reported in the literature, as some studies refer to OA, and others refer to DJD. Although the terms OA and DJD are sometimes used interchangeably, OA refers specifically to a slowly progressive disorder of diarthrodial synovial joints, involving degradation of articular cartilage, osteophyte formation and bone remodeling (Bennett, Zainal Ariffin et al. 2012, Clarke, Mellor et al. 2005, Godfrey 2005, Johnston 1997, Lascelles, Henry III et al. 2010). Degenerative joint disease is a broader term that subsumes OA and also includes pathology of non-synovial joints (*e.g.*, spondylosis of intervertebral joints), and degenerative joint lesions that are not part of OA, such as soft

tissue mineralization and enthesophytes (Bennett, Zainal Ariffin et al. 2012, Clarke, Mellor et al. 2005, Kranenburg, Meij et al. 2012, Lascelles 2010, Ryan, Lascelles et al. 2013). While both OA and non-OA DJD can cause clinical signs of pain and disability, the degree to which each contributes to specific clinical signs has not been elucidated. Because the research conducted as part of this thesis focused on feline OA, the following discussion will focus primarily on what is known regarding the latter, and on appendicular joint DJD where reports do not distinguish between the two.

1.3.2 Epidemiology

1.3.2.1 Prevalence and disease patterns

Early studies of feline OA prevalence consisted of retrospective evaluations based on convenience samples of radiographs taken for a variety of reasons. They were therefore unable to examine all joints, and all radiographic views of joints, for signs of OA, in each cat (Clarke, Mellor et al. 2005, Godfrey 2005, Hardie, Roe et al. 2002). Findings therefore must be interpreted with caution as they likely under-represent true disease prevalence (Kerwin 2010). One such study reported that 16.5% of cats 0.2 to 18 years had OA and 67.6% had DJD in appendicular joints (Clarke, Mellor et al. 2005); another reported that 22% (Godfrey 2005) of cats had at least one appendicular joint with OA. Yet another study reported that 90% of cats over 12 years of age had radiographic DJD, and 64% of cats had appendicular joint DJD (Hardie, Roe et al. 2002). These studies also reported that co-occurrence of OA in multiple joints, and particularly bilateral involvement of the same joints, was common (Clarke, Mellor et al. 2005, Godfrey 2005).

Two studies prospectively evaluated the prevalence of OA, each in 100 client-owned cats (Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011). One found that, of client-owned cats over 6 years of age, 61% had appendicular OA in at least one joint, and 48% had it in more than one joint; 82% of cats over 14 years of age had OA in at least one appendicular joint (Slingerland, Hazewinkel et al. 2011). Another study assessing cats 6 months to 20 years of age found that 92% (overall) had DJD, 91% with appendicular DJD, with a median of 5 affected joints per cat. Age was significantly associated with the presence

of DJD. It was noted that, despite the high prevalence, overall radiographic DJD severity was fairly low, possibly due to a particularity of the species or to the population studied (Lascelles, Henry III et al. 2010). Both reported bilateral disease to be common, especially for the hip, stifle, elbow, tarsus, shoulder, and carpus (Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011). Another study evaluating macroscopic and histopathologic lesions of elbow joints in a sample of adult cats found that cartilage damage associated with DJD tended to be bilateral and of a similar degree of severity on both sides (Freire, Meuten et al. 2014).

Possible reasons for the differences in reported disease prevalence include actual regional population differences (*e.g.*, genetics, environmental risk factors (Lascelles, Henry III et al. 2010)), underestimation of prevalence in convenience samples, as discussed above (Kerwin 2010), and differences in criteria used for evaluating radiographs. Because DJD is a more inclusive radiographic diagnosis than OA (Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011) it is expected that the prevalence of the former would be greater than that of the latter. In addition, only a fair correlation has been reported between the presence of osteophytes and joint associated mineralization and cartilage damage (Freire, Robertson et al. 2011). Because ridges, grooves, roughening of the articular surface, and cartilage loss are not visible radiographically (Bennett, Zainal Ariffin et al. 2012), radiographic studies likely underestimate true disease prevalence.

1.3.2.2 Joints affected

The two prospective radiographic prevalence studies above generally concurred that the appendicular joints of cats that are most commonly affected by OA are (from most to least frequent): the hip, tarsus, and elbow; one also found a high prevalence of OA (mild severity) in the shoulder (Slingerland, Hazewinkel et al. 2011), while the other found stifle DJD to be common (Lascelles, Henry III et al. 2010). The findings of other radiographic studies also support the high prevalence of hip and elbow OA (Clarke, Mellor et al. 2005, Clarke and Bennett 2006, Godfrey 2005). The elbow has been reported to be the most severely affected appendicular joint, radiographically (Hardie, Roe et al. 2002, Lascelles, Henry III et al. 2010). In one study evaluating necropsy samples for joint pathology, macroscopic elbow DJD was found to be very common (present in 73.3% cats) (Freire, Meuten et al. 2014), supporting the

findings of the radiographic studies. Another report found the elbow and stifle to be the joints with the most severe cartilage damage, compared with the tarsus and hip (Freire, Robertson et al. 2011).

1.3.2.3 Etiology and risk factors

Osteoarthritis is a slowly progressive pathological syndrome for which specific etiopathogenesis is unknown and no curative treatment exists (Johnston 1997). Although it is distinct from inflammatory arthritis, and typically considered non-inflammatory, it is associated with a low-grade, nonpurulent inflammation of variable degree (Johnston 1997). It is generally considered to arise either due to trauma from abnormal forces (*e.g.*, fracture, subluxation) acting on a normal joint, or from normal forces acting on an abnormal joint (*e.g.*, developmental abnormalities such as hip dysplasia) (Johnston 1997, Kerwin 2010). The most common form of OA in cats is presumed to be primary, *i.e.*, idiopathic in origin, arising from normal "wear and tear" in the absence of a predisposing condition (Bennett, Zainal Ariffin et al. 2012, Kerwin 2010). One study found that 71% of cats with OA had no detectable underlying radiographic or historical cause and concluded that it was primary/idiopathic in those cases (Clarke and Bennett 2006). Another found no association between lifestyle and DJD (Lascelles, Henry III et al. 2010). However, it is difficult to establish idiopathic origin with certainty, as it is a diagnosis based on exclusion, and joint trauma and other underlying susceptibilities may not be recognized or noted in the animal's history (Bennett, Zainal Ariffin et al. 2012). The classification of OA as primary should therefore be made with caution (Bennett, Zainal Ariffin et al. 2012).

Secondary OA may arise following injury (*e.g.*, cranial cruciate ligament failure (Boyd, Müller et al. 2005)) or in association with other predisposing conditions (Bennett, Zainal Ariffin et al. 2012). The latter include several disorders that tend to cause characteristic severe and/or systemic signs, such as those directly causing articular cartilage degradation (mucopolysaccharidosis and Scottish fold osteochondrodysplasia (Allan 2000)), hypervitaminosis A (Allan 2000, Kerwin 2010), and infectious (*e.g.*, bacterial, viral fungal) or immune-mediated causes (*e.g.*, rheumatoid arthritis, systemic lupus erythematosus, idiopathic polyarthritides, progressive proliferative polyarthropathies) (Allan 2000). In addition,

endocrine disorders such as acromegaly and diabetic neuropathy, and developmental dysplasias such as hip dysplasia, and medial patellar luxation (Loughin, Kerwin et al. 2006) may predispose to OA development. It is not clear to what extent elbow dysplasia, including medial coronoid process disease (Staiger and Beale 2005) and developmental luxation of the radial head, may contribute to OA in cats (Freire, Meuten et al. 2014). Hip dysplasia in cats differs somewhat from that in dogs in that it tends to involve a shallow acetabulum with craniodorsal remodeling, and subluxation is uncommon, as is remodeling of the femoral head and neck (Allan 2000, Keller, Reed et al. 1999). The prevalence of hip dysplasia in cats has been reported as 6.6%, with some breed predilections (*e.g.*, 21.1% prevalence has been reported in the Maine Coon) (Keller, Reed et al. 1999). One study found hip dysplasia to be associated with hip OA in only 20% of cases (Clarke and Bennett 2006). Another report found that hip dysplasia and trauma such as hyperextension, fractures and luxation/subluxation underlay OA in 13/29 cases (Clarke, Mellor et al. 2005); yet another found predisposing conditions (including hip dysplasia, bacterial osteomyelitis, fracture, neoplasia) in only 11% of cats with radiographic OA (Godfrey 2005).

With respect to primary or idiopathic disease, age is the most important risk factor for OA or DJD in cats (Bennett, Zainal Ariffin et al. 2012, Clarke, Mellor et al. 2005, Godfrey 2005, Lascelles, Henry III et al. 2010, Ryan, Lascelles et al. 2013, Slingerland, Hazewinkel et al. 2011). Only young cats (<7 years) lacked macroscopic cartilage changes in a study examining necropsy samples for macroscopic and histopathologic lesions of elbow joints (Freire, Meuten et al. 2014). No association of sex with OA has been demonstrated (Godfrey 2005, Slingerland, Hazewinkel et al. 2011). It is not clear whether weight or body condition score (BCS) influences OA development; one study found cats with elbow OA were heavier (and had a higher BCS) than those without (Ryan, Lascelles et al. 2013), but others have found no association (Clarke, Mellor et al. 2005, Slingerland, Hazewinkel et al. 2011), or a negative association (*e.g.*, with axial DJD severity (Lascelles, Henry III et al. 2010)). The effects of weight on OA development are difficult to elucidate in part because cats' weight tends to be highest in middle age and to decline thereafter (Lascelles, Henry III et al. 2010, Lund, Armstrong et al. 2005, Slingerland, Hazewinkel et al. 2011), and weight at diagnosis may not reflect past weight or BCS.

1.3.3 Articular structural changes associated with feline osteoarthritis

Osteoarthritis affects all joint tissues, including articular cartilage, subchondral bone, synovium, joint capsule, ligaments and muscle (Johnston 1997). Disruption of normal cartilage structure and homeostasis begins as fibrillation of the superficial articular cartilage, resulting in alterations in the composition of the cartilage matrix (Freire, Meuten et al. 2014, Freire, Robertson et al. 2011, Johnston 1997). Cartilage damage was best predicted by age in one study (Freire, Robertson et al. 2011). These changes, in addition to changes in the subchondral bone, lead to abnormal stresses that produce further cartilage damage (fissures, thinning/loss of cartilage potentially exposing subchondral bone, or thickening of cartilage) (Freire, Meuten et al. 2014, Freire, Robertson et al. 2011, Johnston 1997). Subchondral bone pathology may also exist in the absence of overlying cartilage abnormalities (Ryan, Lascelles et al. 2013). This may include remodeling and increased density of subchondral bone, as well as occasional subchondral osseous cyst like lesions (Allan 2000). Free fragments of cartilage produce inflammation; this results in increased synovial fluid production (joint effusion) and protein content, but poorer lubrication (Johnston 1997). Synovial effusion and periarticular thickening appear to be less common in cats than in dogs (Allan 2000, Bennett, Zainal Ariffin et al. 2012). Osteophytes tend to develop at the joint periphery (junction of synovium, periosteum and perichondrium), and the joint capsule thickens and becomes more vascular in OA (Johnston 1997). Narrowing or even collapse of the joint space may occur in advanced disease (Allan 2000). Pain associated with the disease causes disuse, which leads to muscle atrophy, and increased stress on joint capsule, ligaments, and articular cartilage (Johnston 1997).

Intra-articular ossific bodies are more common in cats than in dogs with OA (Allan 2000). Soft tissue mineralization (*e.g.*, of the joint capsule, menisci, and ligaments such as the cranial cruciate ligament) is a prominent feature of feline OA (*e.g.*, in the elbow), in addition to the formation of enthesophytes and osteophytes, and increased size of sesamoid bones (Allan 2000, Bennett, Zainal Ariffin et al. 2012, Lascelles, Henry III et al. 2010). However, it may also occur in the absence of disease (Allan 2000, Bennett, Zainal Ariffin et al. 2012). Synovial osteochondromatosis also occurs in feline arthritic joints and involves formation of

cartilage intra- or extra-articularly *via* synovial metaplasia; it may or may not be radiographically apparent, depending on the degree of ossification (Allan 2000, Bennett, Zainal Ariffin et al. 2012). One study found this to be the mechanism for the finding of osteochondral fragments free in the elbow joint or attached to the synovium, rather than fragmented medial coronoid process or osteochondritis dissecans, as in the dog (Freire, Meuten et al. 2014).

1.3.3.1 Radiographic appearance of feline osteoarthritis

Radiographic features of OA include osteophytes, subchondral bone sclerosis, soft tissue mineralization, enthesophytes, effusion, and joint space narrowing (Bennett, Zainal Ariffin et al. 2012, Clarke, Mellor et al. 2005, Godfrey 2005, Guillot, Moreau et al. 2013, Gunew, Menrath et al. 2008). Bone marrow edema-like lesions have also been reported in coxofemoral OA (Guillot, Moreau et al. 2012). Capsular and extra-capsular mineralizations (*e.g.*, increased prominence of sesamoid bones) are also included in the diagnosis of appendicular DJD (Bennett, Zainal Ariffin et al. 2012, Freire, Robertson et al. 2011, Slingerland, Hazewinkel et al. 2011). More severe osteophytosis has been reported to be associated with a higher likelihood of subchondral bone sclerosis (Clarke, Mellor et al. 2005). However, radiographically apparent sclerosis may result from changes other than increased density of bone trabeculae, such as superimposition of continuous osteophytes over the articular margin, or of other mineralized structures over the epiphyseal bone (*e.g.*, joint capsule mineralization, osteochondromas) (Bennett, Zainal Ariffin et al. 2012, Clarke, Mellor et al. 2005). Joints with a normal radiographic appearance may have cartilage pathology (Bennett, Zainal Ariffin et al. 2012, Freire, Robertson et al. 2011), or even mild osteophytosis detectable *via* magnetic resonance imaging (MRI) (Guillot, Moreau et al. 2012).

The radiographic appearance of feline DJD differs from that in dogs, particularly with respect to the prevalence of joint associated mineralization and meniscal mineralization (Bennett, Zainal Ariffin et al. 2012, Freire, Robertson et al. 2011). Although in the elbow, hip and tarsus, the radiographic DJD change most associated with cartilage damage was osteophytosis, intra articular mineralization has been reported to be most associated with cartilage damage in the stifle (Freire, Robertson et al. 2011). Opacity associated with joint

effusion, and subchondral erosions-cysts were found to be uncommon in one feline DJD study (Freire, Robertson et al. 2011). Osteophytes tend to be mild (Clarke, Mellor et al. 2005, Freire, Robertson et al. 2011, Hardie, Roe et al. 2002), suggesting that such radiographic signs may be less pronounced in this species (Bennett, Zainal Ariffin et al. 2012).

1.3.4 Clinical osteoarthritis in cats

1.3.4.1 Relationship between radiographic and clinical osteoarthritis

There is a mismatch between clinical and radiographic signs of feline OA (Bennett, Zainal Ariffin et al. 2012, Godfrey 2005, Lascelles, Hansen et al. 2007). This may be due in part to the presence of asymptomatic joint changes as well as to lack of identification of signs (Godfrey 2005). It has been suggested that the bilateral nature of OA in the cat, in particular, may mask its clinical signs (Hardie, Roe et al. 2002). Clinical OA therefore cannot be diagnosed on the basis of radiographic signs alone; it has been recommended that clinical history and signs (*e.g.*, lameness, trouble jumping, behavior change, and pain or decreased joint mobility upon examination), or histopathology should support it (Bennett, Zainal Ariffin et al. 2012, Godfrey 2005, Gunew, Menrath et al. 2008, Lascelles and Robertson 2010).

1.3.4.2 Clinical signs of feline osteoarthritis

1.3.4.2.i Gait changes

Although lameness, along with other gait abnormalities such as a stiff or shuffling gait, has been reported as a sign of feline OA, several authors note that it is not a common finding (Bennett, Zainal Ariffin et al. 2012, Clarke, Mellor et al. 2005, Clarke and Bennett 2006, Godfrey 2005, Gunew, Menrath et al. 2008, Hardie, Roe et al. 2002). One study reported that only 16.7% of cats with radiographic OA were lame (Clarke, Mellor et al. 2005), but thorough orthopedic examination was not performed in all cases in this study, so that subtle clinical signs may have gone undetected. Thirty-two to 43% of cats diagnosed with clinical OA had stiffness or limping in another study (Clarke and Bennett 2006). Interestingly, cats with experimental cranial cruciate ligament transection have been reported not to show lameness one year later (Suter, Herzog et al. 1998). It has been suggested that the relative lack of

prominence of lameness as a clinical sign may be due to frequent bilateral disease, the agility of the species, or a predator-avoidance strategy that causes the cat to mask its pain (Bennett, Zainal Ariffin et al. 2012).

1.3.4.2.ii Behavior and lifestyle changes

Behavior and lifestyle changes are thought to be important signs of feline OA (Bennett, Zainal Ariffin et al. 2012). Owner report therefore plays a key role in the detection and monitoring of feline OA (Bennett, Zainal Ariffin et al. 2012). The most commonly reported changes involve mobility and activity. Mobility-related signs relate to: willingness to jump, jump height, difficulty jumping, use of stairs, stiffness, hind limb weakness, and difficulty stretching and rising after rest (Clarke and Bennett 2006, Godfrey 2005, Lascelles, Hansen et al. 2007, Slingerland, Hazewinkel et al. 2011, Zamprogno, Hansen et al. 2010). Activity has been assessed both objectively (see below) and subjectively, with subjective signs of activity level involving owner ratings of sleeping, running, playing, and hunting (Bennett and Morton 2009, Clarke and Bennett 2006, Godfrey 2005, Lascelles, Hansen et al. 2007, Zamprogno, Hansen et al. 2010). In addition to mobility and activity, changes in self-care and mood have been reported in OA cats. Signs relating to self-care include: changes in appetite, weight loss, changes in elimination behavior (*e.g.*, inappropriate elimination or elimination just over the edge of the litter box), and changes in self-grooming (*e.g.*, reflected by coat condition) and scratching behavior (Bennett and Morton 2009, Slingerland, Hazewinkel et al. 2011, Zamprogno, Hansen et al. 2010). Signs of changes in mood that may be associated with OA include: aggression toward or avoidance of household members (human or animal) and seclusion (Bennett and Morton 2009, Zamprogno, Hansen et al. 2010). Interestingly, in a study evaluating indices of quality of life (QOL) in cats, a large proportion (60%) of items listed by owners as being important to QOL were "inactive" (*e.g.*, sleeping, being petted) rather than "active" (*e.g.*, running, playing with toys); this was the case for cats with DJD as well as those without, suggesting that activity alone may not provide a complete picture in DJD assessment and that inactive behaviors should be considered in evaluating therapeutic interventions, as possible measures of the affective dimension of pain (Benito, Gruen et al. 2012).

It should be noted that the reported signs described above are based on studies of cats diagnosed both with OA and with DJD, and therefore may reflect appendicular or axial (including spondylosis) disease (Bennett, Zainal Ariffin et al. 2012, Kranenburg, Meij et al. 2012). It is not possible to localize the particular joints affected, based on these behavioral and lifestyle changes.

1.3.4.3 Physical examination findings in feline osteoarthritis

Physical examination of the cat for OA is challenging. Gait can be difficult to assess in this species (due to lack of cooperation with walking in a straight line), and cats may object to palpation and manipulation of the joints even in the absence of pain (Bennett and Morton 2009, Bennett, Zainal Ariffin et al. 2012, Clarke and Bennett 2006, Kerwin 2012, Kranenburg, Meij et al. 2012, Slingerland, Hazewinkel et al. 2011). Palpation may therefore overestimate clinical OA (Clarke and Bennett 2006, Lascelles, Dong et al. 2012), although it should be considered that the mismatch between pain on palpation and radiographic signs could be due to the presence of morphological (*e.g.*, cartilage) damage without radiographically detectable lesions (Freire, Robertson et al. 2011, Lascelles, Dong et al. 2012). Conversely, a lack of synovitis in the presence of severe cartilage damage could explain a lack of pain upon palpation in radiographically affected joints (Freire, Meuten et al. 2014).

Palpable abnormalities such as joint thickening, synovial effusion, crepitus, and reduced range of motion are less obvious in the cat than in the dog (Bennett, Zainal Ariffin et al. 2012). One study reported that the hip and shoulder were difficult to assess for periarticular thickening, but that 67% of other OA joints demonstrated mild thickening; reduced range of motion was detected in only 6% of OA joints, none showed crepitus, and synovial effusion was seldom obvious (Clarke and Bennett 2006). Sensitivity and positive predictive value of joint palpation and manipulation for radiographic DJD has been reported to be low generally, but sensitivity was highest for pain (ranging from 12 to 50% for axial segments and 0 to 67% for appendicular segments), and positive predictive value was highest for thickening (0 to 90%) and crepitus (0 to 86%) of the appendicular joints (Lascelles, Dong et al. 2012). The appendicular joint for which palpation findings had the best sensitivity to DJD was the elbow (ranging from 21 to 67% for pain, thickening, crepitus, or effusion in the study by Lascelles *et*

al., and 71% for overall abnormal clinical examination findings in the study by Slingerland *et al.*) (Lascelles, Dong et al. 2012, Slingerland, Hazewinkel et al. 2011). Goniometry (joint range of motion measurement) has been found to be reliable, with no substantial measurement differences produced by sedation (Jaeger, Marcellin-Little et al. 2007, Lascelles, Dong et al. 2012). Because specificity and negative predictive values tend to be higher, it has been suggested that an absence of findings, particularly pain and decreased range of motion (*e.g.*, measured *via* goniometry), upon joint palpation and manipulation may be useful clinically for ruling out DJD (Lascelles, Dong et al. 2012, Slingerland, Hazewinkel et al. 2011). One study found that cats with higher temperament scores ("unfriendly") upon examination had higher radiographic DJD and pain scores, and concluded that a worse temperament score in DJD cats suggests pain and has potential for use in assessment of analgesic efficacy (Lascelles, Dong et al. 2012). A second study also reported that reaction to handling was negatively correlated with objectively measured activity, providing some support for this suggestion (Benito, Hansen et al. 2013). However, no studies to date have directly examined the contributions of different factors (*e.g.*, OA or non-OA pain, anxiety or fear) to feline response to physical examination.

1.3.4.4 Comorbidities in feline osteoarthritis

Because OA prevalence increases with age in cats (Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011), and older animals are susceptible to other geriatric diseases, cats with OA may be at risk for comorbidities. Chronic kidney disease (CKD) is common in cats and has been reported to have a significant concurrence with DJD (Marino, Lascelles et al. 2014). Suggested reasons for this include: 1) that behavioral changes reported to be associated with DJD may also arise due to CKD (*i.e.*, in cats with radiographic signs of DJD, decreased activity and interaction with family may result either from DJD or CKD), or 2) that the two diseases share a common etiology (*e.g.*, inflammatory or immune-mediated) (Marino, Lascelles et al. 2014). One report also proposed that nonregenerative anemia may result from chronic inflammation in feline OA (Clarke and Bennett 2006).

1.3.5 Measures of pain and disability attributable to feline osteoarthritis

Cats are notoriously difficult to assess with respect to pain (Lascelles 2010, Zamprogno, Hansen et al. 2010). However, in the last decade or so, there has been increasing interest in the problem of feline pain identification and measurement, with numerous investigations of pain signs and outcome measures both for acute (*e.g.*, post-operative) and for chronic conditions such as OA (Merola and Mills 2016). That said, measures do not exist for evaluation of all causes of feline pain, and few have undergone thorough validation (Merola and Mills 2016).

1.3.5.1 Objective measures in feline osteoarthritis

A number of objective measures have been evaluated for OA pain. Telemetric locomotor activity monitoring (AM), using collar-mounted devices, has been shown to detect activity increases in response to treatment of OA cats with an NSAID, meloxicam (Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2007, Monteiro, Klinck et al. 2016), a therapeutic diet (Lascelles, DePuy et al. 2010), a feline-specific anti-nerve growth factor (Gruen, Thomson et al. 2016), and tramadol (Monteiro, Klinck et al. 2016), but is subject to substantial inter-cat variation, making it less useful for distinguishing OA pain presence/severity between individual cats (Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2007, Monteiro, Klinck et al. 2016). A recent report evaluating functional data analysis for AM found that patterns of activity (unlike the previously reported use of mean activity by period) did distinguish between OA and non-OA cats, with higher peaks and troughs in non-OA than in OA cats (Gruen, Alfaro-Córdoba et al. 2017). This suggests further potential for this method of AM analysis. Evaluation of ground reaction forces using either a force plate (Corbee, Maas et al. 2014, Suter, Herzog et al. 1998) or a pressure-sensing walkway has also been described in cats. Peak vertical force (PVF) measured by the latter distinguishes OA from non-OA cats (Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013), and the most affected limb (based on PVF) tended to be that most severely affected radiographically (Guillot, Moreau et al. 2013). However, PVF does not consistently detect treatment effects (Guillot, Moreau et al. 2013, Monteiro, Klinck et al. 2016). Tests of altered pain processing, *i.e.* central sensitization,

such as the von Frey anesthesiometer-induced paw withdrawal threshold (PWT), which tests for allodynia, and response to mechanical temporal summation (RMTS), also distinguish OA from non-OA cats (Guillot, Taylor et al. 2014). Response to mechanical temporal summation has been found to detect responses to treatment with tramadol (Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017); neither it nor PWT are responsive to meloxicam (Guillot, Moreau et al. 2013, Monteiro, Klinck et al. 2016), consistent with the mechanisms of action of these medications. The latter involves a primary anti-inflammatory effect producing analgesia, for meloxicam, while tramadol creates analgesia through opioid agonist and monoaminergic reuptake inhibitory effects (Beakley, Kaye et al. 2015). Peak vertical force, PWT, and AM have been found to be reliable (*i.e.*, repeatable) (Guillot, Moreau et al. 2013, Moreau, Guillot et al. 2013). Thermography is another modality that has been described for evaluation of musculoskeletal pain in cats. This is a noninvasive technique that uses measurement of infrared radiation to generate a pictorial representation of surface (skin) temperature (Turner 2001); its use is common in equine lameness evaluation, and has also been reported in canine joint disease (Infernuso, Loughin et al. 2010, McGowan, Loughin et al. 2015, Turner 2001). However, correlations with palpation findings suggestive of pain, but not subjective pain assessments, make its usefulness for evaluating musculoskeletal pain in cats unclear at this time (Vainionpää, Raekallio et al. 2013).

1.3.5.2 Subjective measures in feline osteoarthritis

Arguments for the use of subjective pain measures, *i.e.*, pain scales, in feline OA include the importance of lifestyle changes (in the home) as signs of the disease, as discussed above, and the fact that specialized equipment, technical skill, and cat training are not needed for clinical application of such measures, *vs.* the objective measures above. However, a substantial hurdle to their use is the need for validation studies to show that they reliably measure feline OA pain and functional impairment, in the target population, as described in section 1.2. Patient-reported outcome measures are reportedly effective in monitoring human outpatient populations with specific health conditions (Boyce and Browne 2013); however, lack of self-report in animals means that a proxy is needed, increasing the potential error of measurement (Merola and Mills 2016). Studies comparing self-report (gold standard) to proxy

evaluation in human patients have found healthcare providers' pain assessments to be only moderate to good, with poorer accuracy for higher levels of pain, in older patients, and for practitioners with < 4 or > 10 years of experience (Ruben, van Osch et al. 2015). It is recommended that the person most familiar with the human patient provide proxy ratings, where possible (Herr, Coyne et al. 2011). Generally, healthcare providers such as doctors and nurses are more likely to underestimate, and family members may be more likely to overestimate, human patients' pain (with the exception of parents of children with intellectual disabilities, who are more likely to underestimate their child's pain) (Herr, Coyne et al. 2011). The precise implications of these findings for proxy assessment of animal pain are not clear; however, they do provide some insight regarding potential sources of error.

At the start of this project, no validated, standardized pain scales had been reported for assessment of feline OA-related pain and functional impairment (Lascelles 2010). The one subjective tool for which there was preliminary validation was the CSOM. This is a personalized scale in which the veterinarian and the pet owner work together to identify a short list (a total of three activities was recommended) of the cat in question's activities that appear affected by OA, and to grade each one based on how severely the activity is affected (Lascelles, Hansen et al. 2007). This scale therefore requires that a diagnosis already have been made; it has shown inconsistent ability to detect therapeutic effects of meloxicam (Gruen, Griffith et al. 2015, Lascelles, Hansen et al. 2007), though it did detect analgesia due to feline-specific anti-nerve growth factor (Gruen, Thomson et al. 2016). A handful of other feline OA pain scales have been reported more recently, with varying degrees of validation. The first such scale was described by Bennett and Morton in 2009 (Bennett and Morton 2009). The authors noted the benefits of assessing signs of pain specific to the feline patient, but proposed a standardized assessment, rather than the CSOM, as being less labor intensive. This scale was developed with four domains, mobility, activity, grooming, and temperament; specific examples of behaviors to consider when evaluating these domains were provided. Partial validation was performed *via* a trial of meloxicam, which resulted in improved pain scale scores; however, ability to distinguish treatment from placebo is unknown as there was no control group, nor were comparisons made between OA and non-OA cats.

Another pain scale, the Feline Musculoskeletal Pain Index (FMPI), has undergone substantially more validation (Benito, Depuy et al. 2013, Benito, Hansen et al. 2013, Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Zamprogno, Hansen et al. 2010). This scale has shown good results for readability, test-retest and internal consistency reliability, and ability to discriminate OA from non-OA cats (Benito, Depuy et al. 2013). Evidence of validity based on relations with objectively measured activity (AM; convergent, construct validity) and ability to detect response to treatment was initially inadequate (Benito, Hansen et al. 2013), but convergence with AM improved following scale refinements (Gruen, Griffith et al. 2015). The FMPI has recently been found to detect therapeutic effects of meloxicam (Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015), but not of feline-specific anti-nerve growth factor (Gruen, Thomson et al. 2016).

Although the use of simple pain scales (*e.g.*, VAS, SDS) in assessing feline musculoskeletal pain has been reported (Carroll, Narbe et al. 2008, Clarke and Bennett 2006, Gunew, Menrath et al. 2008), these have not been validated for feline OA pain and functional impairment. Their ability to measure these accurately and reliably is therefore unclear.

1.3.6 Treatments for feline osteoarthritis

Treatment options for feline OA are somewhat limited. The only treatment approved for long-term use in feline musculoskeletal disease is meloxicam, an NSAID, and that only in Europe and Australia (Kerwin 2010, Lascelles and Robertson 2010). The following section reviews what is known regarding treatments used in, or proposed for feline OA.

1.3.6.1 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs are the cornerstone of treatment of OA in many species (Bennett, Zainal Ariffin et al. 2012). In the cat, the therapeutic agent with the most support for its efficacy in OA is meloxicam, with reported improvements in subjectively assessed mobility (*e.g.*, jumping, lameness/stiffness), activity level, and mood/demeanor, as well as in objectively measured activity (AM) (Bennett and Morton 2009, Clarke and Bennett 2006, Gruen, Griffith et al. 2014, Guillot, Moreau et al. 2013, Kerwin 2010, Lascelles, Hansen

et al. 2007). However, concerns exist over the safety of long-term administration of an NSAID to cats, particularly due to potential for delayed metabolism in this species (Bennett, Zainal Ariffin et al. 2012, Lascelles 2010, Lascelles, Court et al. 2007), as well as the potential nephrotoxicity of these drugs and the high prevalence of CKD in cats, especially in those with DJD (Marino, Lascelles et al. 2014). Reported adverse events in meloxicam treatment of OA include gastrointestinal signs (*e.g.*, vomiting) and both acute renal failure and stable increases in renal analytes (Benito, Hansen et al. 2013, Bennett and Morton 2009, Clarke and Bennett 2006, Gunew, Menrath et al. 2008, Lascelles, Hansen et al. 2007). That said, one study evaluating the safety of long-term meloxicam administration found that CKD did not progress more rapidly in cats receiving meloxicam than in not receiving it (Gowan, Baral et al. 2012), and another study reported that it was well tolerated in cats treated for a mean duration of 5.8 months (Gunew, Menrath et al. 2008). The licensed dose for long-term use is 0.05 mg/kg daily, by mouth, but it has been recommended that the lowest effective dose be used (Bennett, Zainal Ariffin et al. 2012, Sparkes, Heiene et al. 2010), and efficacy in chronic musculoskeletal disease has been reported at doses as low as 0.01 to 0.03 mg/kg per day (Guillot, Moreau et al. 2013, Gunew, Menrath et al. 2008). Other NSAIDs have been proposed for chronic feline OA pain (*e.g.*, ketoprofen, tolfenamic acid, robenacoxib)(Bennett, Zainal Ariffin et al. 2012, Lascelles and Robertson 2010) but are not licensed for this use; robenacoxib has been found not to have significant adverse effects when administered long-term in healthy cats (King, King et al. 2016). Careful dose measurement is recommended when administering NSAIDs to cats, along with administration with food, avoidance of use in dehydrated or anorexic animals, and monitoring of serum renal analytes, to minimize the impact of any adverse effects (*e.g.*, gastrointestinal signs such as vomiting, acute renal failure) (Sparkes, Heiene et al. 2010).

1.3.6.2 Other analgesics

There is limited published evidence for the use of other analgesics in feline OA. However, recent studies have reported efficacy for a feline-specific nerve growth factor antibody (anti-NGF) (Gruen, Thomson et al. 2016), and tramadol (Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017). Treatment of client-owned cats with DJD with anti-NGF

resulted in increased objectively measured activity (AM), and in improvements in CSOM scores (Gruen, Thomson et al. 2016). Tramadol is an analgesic agent with a mixed and incompletely understood mechanism of action, including μ-opioid receptor activation, serotonin and norepinephrine reuptake inhibition, as well as G-coupled protein receptor and ion channel effects (Grond and Sablotzki 2004, Minami, Ogata et al. 2015). It increased objectively measured activity (AM) and PVF, and decreased nociceptive hypersensitivity quantified by RMTS, when administered alone to cats with OA (Monteiro, Klinck et al. 2017); when given with an oral transmucosal meloxicam spray, it also decreased nociceptive hypersensitivity measured *via* RMTS (the latter was unaffected by meloxicam treatment alone) (Monteiro, Klinck et al. 2016). Other proposed analgesics for chronic OA treatment include opioids (*e.g.*, buprenorphine), gabapentin, amantadine, and amitriptyline (Bennett, Zainal Ariffin et al. 2012, Lascelles and Robertson 2010); however, their long-term use in OA has not been systematically evaluated.

1.3.6.3 Other treatment modalities

Non-pharmaceutical, disease-modifying osteoarthritis agent therapies that may be of benefit in feline OA include oral glucosamine and chondroitin supplements, injectable pentosan polysulfate (pentosan PS), and dietary supplementation with omega-3 fatty acids. One study comparing glucosamine to meloxicam treatment in cats with OA found no significant improvement in the glucosamine group (Sul, Chase et al. 2014). No systematic assessment of pentosan PS in feline OA has been conducted. A therapeutic trial of a diet formulated with high levels of omega-3 fatty acids (eicosapentaenoic and docosahexaenoic acids), and enriched with green-lipped mussel extract, glucosamine, and chondroitin sulfate, found that it increased objectively measured activity (AM) (Lascelles, DePuy et al. 2010). Environmental modification and modulation of activity have also been recommended for management of feline OA. The former involves maintaining access to preferred locations (*e.g.*, arrangement of furniture to provide "steps" to beds and windows, and ensuring easy access to the litterbox). Modulation of activity can be accomplished both *via* such environmental changes (facilitating the cat's movements about the home) and direct encouragement (*e.g.*, stimulation of gentle activity through play) (Lascelles and Robertson 2010).

1.4 Translational pain assessment: Could natural animal models be the missing link?

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1.4.1 Abstract

Failure of analgesic drugs in clinical development is common. Along with the current "reproducibility crisis" in pain research, this has led some to question the use of animal models. Experimental models tend to comprise genetically homogeneous groups of young, male rodents in restricted and unvarying environments, and pain-producing assays that may not closely mimic the natural condition of interest. In addition, typical experimental outcome measures using thresholds or latencies for withdrawal may not adequately reflect clinical pain phenomena pertinent to human patients. It has been suggested that naturally-occurring disease in veterinary patients may provide more valid models for the study of painful disease. Many painful conditions in animals resemble those in people. Like humans, veterinary patients are genetically diverse, often live to old age, and enjoy a complex environment, often the same as their owners'. There is increasing interest in the development and validation of outcome measures for detecting pain in veterinary patients; these include objective (*e.g.*, locomotor activity monitoring, kinetic evaluation, quantitative sensory testing, bio-imaging) and

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subjective (*e.g.*, pain scales, quality of life scales) measures. Veterinary subject diversity, pathophysiological similarities with humans, and diverse outcome measures could yield better generalizability of findings and improved translation potential, potentially benefiting both humans and animals. The Comparative Oncology Trial Consortium in dogs has pawed the way for translational research, surmounting the challenges inherent in veterinary clinical trials. This review describes numerous conditions similarly applicable to pain research, with potential mutual benefits for human and veterinary clinicians, and their respective patients.

1.4.2 Introduction

Major goals of pain research are improving our understanding of pain pathology and identifying novel molecular therapeutic targets for better clinical pain management. Despite huge scientific and technological advances, and tremendously increased research & development costs, drugs are more likely to fail in clinical development today than they were in the 1970s (Bowen and Casadevall 2015, Scannell and Bosley 2016). The credibility of efficacy data obtained from animal disease models has lately been called into question, in biomedical research in general (McGonigle and Ruggeri 2014, Scannell and Bosley 2016, Tsukamoto 2016) and in the pain field specifically (Blackburn-Munro 2004, Mogil 2009, Mogil and Crager 2004, Mogil, Davis et al. 2010, Quessy 2010, Stephenson and Arneric 2008). Scientific research also faces a "reproducibility crisis", which may be a contributing factor in the translational crisis. Failure rates in the clinical phase are around 90-95% (Arrowsmith 2012, Hay, Thomas et al. 2014, Regan, Hockenhull et al. 2014), due in part to the challenges of interpreting animal model data. The predictability of basic research varies depending on the understanding and complexity of disease biology: while for therapeutics targeting infectious diseases, success rates are high, for diseases involving complex mechanisms, such as neurological diseases and cancer, they can be as low as 2.3% (Hay, Thomas et al. 2014). For pain studies, the likelihood of eventual FDA approval of a drug entering Phase I studies has been reported at 10.7% (Hay, Thomas et al. 2014). Some have blamed animal models for these translational difficulties. Indeed, a lack of tangible benefit over a long enough period of time could lead one to question both the commitment of substantial funding to, and the ethics of, animal use for this research (Quessy 2010,

Tsukamoto 2016). However, the lack of success is almost certainly also associated with other factors, and both the initial compound development and *in vitro* screening programs should be examined, as well as the animal models used.

The main challenges of animal models are not only recreating disease conditions but also defining measurable and clinically translatable efficacy parameters. For a pain model to be valid, it should encompass key elements of the human pain experience and measure the pertinent aspects of that experience. Given that an animal model consists of a subject, a method of pain induction (*i.e.*, an assay), and an outcome measure(s) (Mogil 2009), each of these components should reflect as closely as possible the clinical condition to which the results are to be applied. Some proposed strategies for improving model face and predictive validity include the use of so-called "natural" animal models of painful disease (*i.e.*, veterinary patients) (Brown, Boston et al. 2008, Mogil 2009, Quessy 2010, Rice, Cimino-Brown et al. 2008), and employment of non-evoked outcome measures representative of important clinical pain phenomena (Mogil and Crager 2004, Whittaker and Howarth 2014). The purpose of this review is to examine how the modeling of human pain using animals might be improved, by considering how aspects of the model may influence representation of the pain experience and its interpretation. To what extent are experimental models representative, and natural models consistent with, the pain condition of interest? How do methods of pain evaluation compare between these types of animal models? Finally, we will discuss limitations and practical aspects of studying natural animal models.

1.4.3 Modeling the human pain experience

1.4.3.1 Experimental animal models

Similarities in the neuroanatomy and physiology of pain across mammalian species, as well as evolutionary evidence, argue for parallel pain experiences in humans and animals (Sneddon, Elwood et al. 2014). However, many authors have noted limitations of experimental models (Blackburn-Munro 2004, Le Bars, Gozariu et al. 2001, Mogil 2009, Mogil, Davis et al. 2010, Negus, Vanderah et al. 2006) that may inhibit extrapolation of findings to humans. For instance, clinical pain patients are often middle-aged or older, and a clear majority are women;

the persistent use of young, male, rodent subjects, although less expensive and more convenient for the experimenter, neglects the demonstrated modulatory effects of organismic factors on pain and analgesic responses (Mogil, Davis et al. 2010). It is usually argued that restriction of assays to a single strain, age and sex reduces variability and so increases the likelihood of detection of a drug effect. However, this approach means the study population is a poorer representation of the target population because of these restrictions. Use of factorial experimental designs, to incorporate, for example, genotype, sex, and age can provide more information without increasing the numbers of animals required for the study (Shaw, Festing et al. 2002). Additionally, neuroanatomical variations between and within species may affect test responses and apparent pharmacological efficacy, yielding species- (Le Bars, Gozariu et al. 2001) or strain-specific results (Mogil 2009). Environments which are confining, homogenous and unvarying, or otherwise inappropriate, or insufficient habituation periods, may also affect neurophysiology (Gonder and Laber 2007), apparent stress responses, and potentially pain assessment (Otis, Gervais et al. 2016). The result is that mere environmental or procedural differences between laboratories (*e.g.*, (Sorge, Martin et al. 2014)) can contribute to discordant research findings and complicate interpretation. In addition, poor reproducibility can arise due to variability in assay methods, particularly since experimental assays may not truly model natural disease processes, with respect to pathophysiology, duration, persistency, severity, *etc*. (Blackburn-Munro 2004, Negus, Vanderah et al. 2006, Rice, Cimino-Brown et al. 2008, Sikandar, Ronga et al. 2013). Incorporating additional species, using naturally-occurring models, as an adjunct to rodent studies, can help address some of these issues.

1.4.3.2 Natural animal models

The potential contribution of naturally-occurring diseases in animals as models of human disease is not new. In 1929, Nobel laureate August Krogh wrote: “For a large number of problems, there will be some animal of choice or a few such animals on which it can be most conveniently studied” (Krogh 1929). Naturally-occurring diseases in companion animals might better reflect the complex genetic, environmental (diverse diets and personal habits), and physiological variation present in humans (Kol, Arzi et al. 2015). This presents both

advantages (a more representative model) and disadvantages (increased complexity in the design and interpretation of studies using these subjects). Effectiveness of new analgesic treatments in canine or feline painful conditions would be anticipated to predict similar benefits in human patients. Using these spontaneous models as part of the drug development process (*e.g.*, as has recently been done in canine osteoarthritis and cancer (Stroud, Dmitriev et al. 2016)) could yield higher "translatability" (see examples in section 3.2 for osteoarthritis; also recent FDA granting of a Fast Track Designation for recombinant HER2-expressing *Listeria monocytogenes* immunotherapy for pediatric osteosarcoma, based on encouraging results, including prolonged survival, in dogs with the disease[9]). However, it also raises major practical issues relating to recruitment of sufficient subjects and this is discussed in more detail below.

Papers discussing specific natural animal models exist for feline interstitial cystitis as a model of visceral pain (Buffington 2001), for feline diabetes mellitus as a model of neuropathic pain (Mizisin, Shelton et al. 2002), for osteoarthritis in many species (McCoy 2015, Moreau, Pelletier et al. 2013), and for various tumors in dogs (Brown, Boston et al. 2009, Leroy and Northrup 2009, Pena, Perez-Alenza et al. 2003, Vail and MacEwen 2000) and cats (Pérez-Alenza, Jiménez et al. 2004, Vail and MacEwen 2000). However, this barely scratches the surface of the huge overlap of veterinary and human painful diseases. See Figure 1.4.1 for some examples of painful veterinary conditions.

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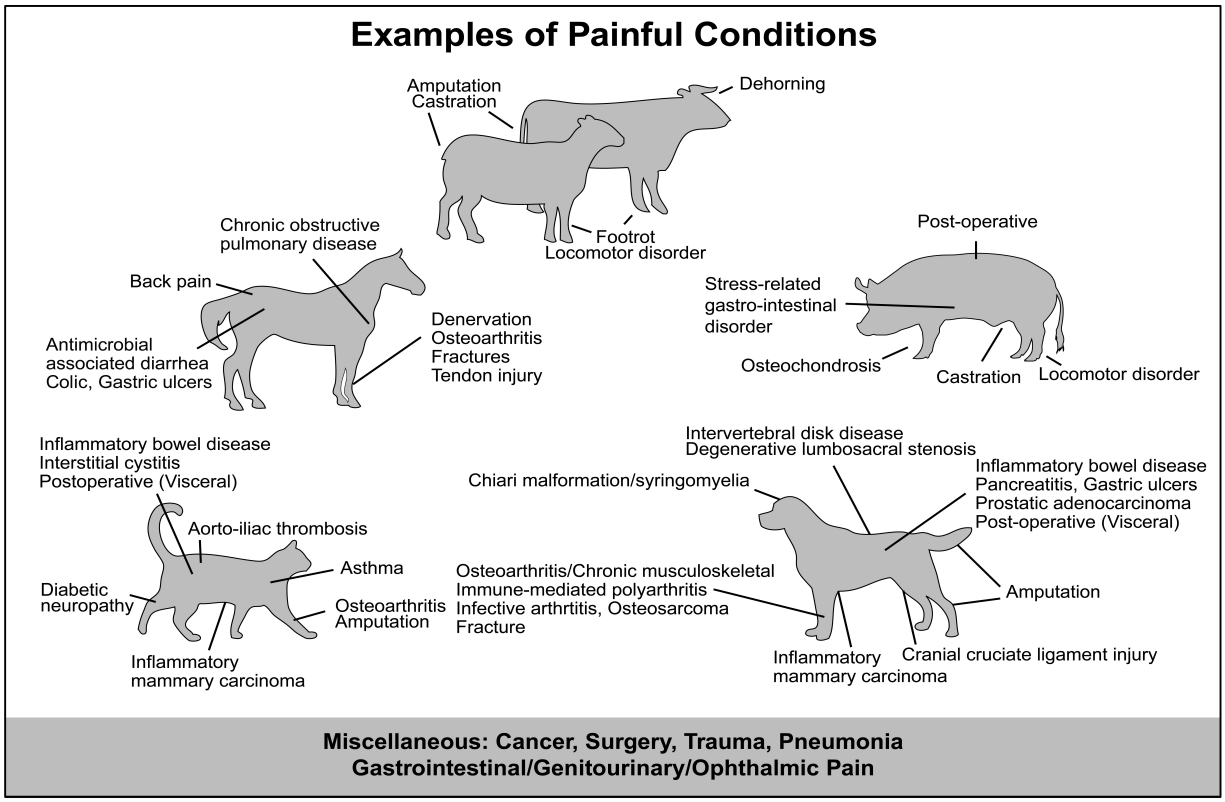
9 https://globenewswire.com/news-release/2016/04/28/834081/0/en/FDA-Grants-Advaxis-Fast-Track-Designation-for-ADXS-HER2-for-Patients-with-Newly-Diagnosed-Non-Metastatic-Surgically-Resectable-Osteosarcoma.html

Figure 1.4.1: Examples of common painful conditions in companion animals.



(© GREPAQ-2017)

The most accessible veterinary patients for study are the commonly kept species, *i.e.,* domesticated animals (pet dogs and cats, and livestock including horse, swine, sheep, and cattle). Sex and age distributions either of particular patient types, or representative of the general veterinary population, might be more similar to those in human medicine. Animals may be castrated/ovariectomized, potentially affecting pain physiology and analgesic responses; however, this may be advantageous in some cases, such as in the study of painful conditions prevalent in post-menopausal women. Both genetically diverse (*e.g.*, veterinary clinic patients) and related (*e.g.*, particular breeds, lineages, siblings, or littermates) populations are available; some conditions also occur within multiple species. These genetically variable subjects might yield more generalizable findings than the inbred rodent strains in most common use in pain research presently (Mogil 2009). Companion animals often share the human environment, exposing them to similar epigenetic, and other risk and

disease-modulating, factors. They also often live to old age, permitting the study of the effects of such factors over the long term, as well as of the secondary effects of pain or painful disease over time.

Causes of spontaneous painful disease in veterinary species are variable (*e.g.*, genetic susceptibility, developmental disease, exposure to environmental or occupational risk factors, traumatic events, immune dysfunction), and sometimes difficult to identify, just as in humans. The pathophysiology may differ between man and animals, even in apparently similar diseases (Bjorling, Wang et al. 2011, Forslund 1997), affecting translation potential, or at least requiring further exploration, but the phylogenetic (sequence homology, metabolism, *etc.*) and physiologic (digestive, skin, nociceptive biochemistry and anatomy, *etc.*) proximity to humans for large animals (swine, dogs, cats) over rodents is well established (Gigliuto, De Gregori et al. 2014, Hoeppner, Lundquist et al. 2014, O'Brien, Johnson et al. 2008). Efforts to facilitate multicenter studies are underway in veterinary medicine. For example, in 2007, the canine Comparative Oncology Trials Consortium emerged within the U.S.A. National Cancer Institute. Comparative oncology between humans and dogs was driven by the concordance between these species with respect to cellular and molecular aspects of bone cancer, lymphoma and bladder cancer. Pet dogs often receive high-quality health care into old age; dog owners are highly motivated both to seek out improved treatment options for canine cancer, and to minimize side effects. The genetic diversity and sharing of similar DNA, physiology, cellular structure and molecular features between dogs and humans has presented cancer researchers with a key opportunity. Dogs not only develop cancers like those in humans, their cancer responds similarly to treatments. Under this auspice, canine clinical trials are revealing new aspects of carcinogenesis and cancer biology, and new diagnostic methods, and are enabling the translation of innovative personalized therapeutics, with accurate predictive safety and efficacy, to human clinical trials (Alvarez 2014, Kol, Arzi et al. 2015, Paoloni and Khanna 2008, Paoloni, Webb et al. 2014). A dozen trials have been completed, some supporting pharmaceutical company decisions to drop or to pursue candidate drugs for human use ((Editorial) 2016). The time has come to establish consensus statements and action plans for identifying naturally-occurring diseases with potential for accelerating pain translation (see below for some examples), as well as for creating opportunities for synergistic

clinical education, platforms for fusing graduate education with clinical studies, and interdisciplinary platforms to facilitate dissemination of translational concepts, and for diverting trans-disciplinary resources to support translational medicine research efforts (Alvarez 2014, Kol, Arzi et al. 2015, Paoloni and Khanna 2008, Paoloni, Webb et al. 2014). Establishment of a companion animal pain trials consortium would provide a framework for this. Crucial to the process will be the ability to measure outcomes accurately and reliably.

1.4.4 Measuring pain in animal models

1.4.4.1 Experimental animal models

Common experimental outcome measures involve the determination of thresholds or latencies for nocifensive (*e.g.*, withdrawal) responses to an increasing or continuous stimulus (*e.g.*, mechanical, thermal, chemical, electrical). Of concern is that these tests may lack specificity for pain, also detecting disagreeable, non-painful stimuli (Le Bars, Gozariu et al. 2001), and that they are insensitive to certain analgesics (*e.g.*, steroid, non-steroidal anti-inflammatory drug (NSAID) (Negus, Vanderah et al. 2006)). In addition, they focus on hypersensitivity, neglecting other symptoms (*e.g.*, spontaneous pain) and subjective or emotional aspects of pain (Mogil and Crager 2004, Vierck, Hansson et al. 2008). Failure to reflect the major clinical phenomena of interest may lead to the erroneous rejection of novel analgesics with potentially clinically relevant effects. The poor positive predictive value of animal pain models (10.7% (Hay, Thomas et al. 2014)) may result from drugs with only a modicum of activity being advanced to clinical trials on the basis of weak positive preclinical data. However, it raises the concern that a high rate of false negative prediction may also exist (Tsukamoto 2016). False positives may also arise due to effects other than analgesia (*e.g.*, sedation, anxiolysis) although incorporation of appropriate additional assays to detect such factors may reduce this problem (Le Bars, Gozariu et al. 2001, Negus, Vanderah et al. 2006). In addition, different types and variations of withdrawal tests (*e.g.*, thermostatic bath *vs.* hot plate *vs.* radiant heat, or different stimulus temperatures) can yield conflicting results (Le Bars, Gozariu et al. 2001) or reflect inter-laboratory variability. A multicenter approach for pre-clinical pain studies could provide robust validation and evidence for reproducibility of

models and outcome measures, by systematically assessing reproducibility and inter-laboratory variability and identifying factors that may be associated with such variability. This has been done recently with burrowing behavior in rats in the complete Freund's adjuvant model across 8 centers and 11 studies (Wodarski, Delaney et al. 2016).

Experimental models rarely employ measures of prominent features of clinical pain, such as spontaneous pain, and affective, motivational and functional changes (Mogil 2009, Mogil, Davis et al. 2010, Rice, Cimino-Brown et al. 2008). This is likely due to challenges such as the need for animal training and the influence of other factors on motivation (*e.g.*, in operant tests (Mogil 2009)), the rarity or lack of sensitivity/specificity for pain of some spontaneous pain-related behaviors (Mogil, Graham et al. 2010), and inter-specific differences in brain anatomy, and the labor, time, cost and need for anesthesia in functional neuroimaging (Murrell and Johnson 2006, Stephenson and Arneric 2008). However, accurate modeling of such effects is likely possible (Mogil, Davis et al. 2010), and there is growing interest in the use of operant tests examining motivational changes related to pain and/or analgesics (*e.g.*, escape responses (Morgan, Carter et al. 2008), thermal preference (Datta, Chatterjee et al. 2010, Morgan, Carter et al. 2008), place preference/avoidance (Boyce-Rustay, Zhong et al. 2010, LaBuda and Fuchs 2000, Otis, Gervais et al. 2016), or reinforcement conflict (Mauderli, Acosta-Rua et al. 2000, Neubert, Widmer et al. 2005)), observation of spontaneous/suppressed behaviors, pain scales, quantitative activity monitoring, kinetic (force plate) and kinematic (angular joint movement) assessments, and evaluation of brain processing of pain (Mogil, Davis et al. 2010, Negus, Vanderah et al. 2006), *e.g.*, *via* electroencephalography (EEG), functional magnetic resonance imaging (fMRI), and positron emission tomography (PET). Brain imaging in unrestrained, awake pet dogs has recently emerged as a practical, reliable (Berns, Brooks et al. 2013) tool in comparative neuroscience (Cook, Brooks et al. 2015). Some recent studies describe the validation of measures assessing spontaneous behaviors and behavioral changes for pain evaluation (*e.g.*, the Grimace Scales, which use characteristics of facial expression in a manner similar to that described in the infant, the Facial Action Coding System (FACS), that measures the individual movements or 'action units' of the face that comprise an expression (Grunau and Craig 1987)). Other measures of spontaneous pain in laboratory rodents evaluate spontaneous locomotion, running wheel activity, gait analysis,

sleep behavior, vocalizations, and specific pain-related behaviors such as burrowing. However, the latter have some limitations, particularly that behaviors may vary depending on the part of the body affected; these manifestations tend to be observed in acute pain, while signs of chronic pain are different and may be less overt, and some behaviors may not distinguish pain from general malaise (Whittaker and Howarth 2014). The Mouse Grimace Scale (MGS, including orbital tightening, nose/cheek bulge, ear/whisker position) has high reliability and accuracy (improving with experience of the observer), but is only useful in pain models of moderate duration (Langford, Bailey et al. 2010); mice do not grimace long-term in neuropathic models. The Grimace Scale has been translated successfully to the rat (chemical and surgical models) (Oliver, De Rantere et al. 2014, Sotocinal, Sorge et al. 2011), and rabbit (ear tattooing) (Keating, Thomas et al. 2012) with the sole modification being the use of nose/cheek flattening instead of bulging. Interestingly, similar positive results were observed in feline pain (ear position and nose/muzzle shape) (Holden, Calvo et al. 2014) and ovine pain (Guesgen, Beausoleil et al. 2016, Marini, Colditz et al. 2015), as well as in bovine spontaneous (Gleerup, Andersen et al. 2015) and experimental (Rialland, Otis et al. 2014), and equine experimental (Gleerup, Forkman et al. 2015) and surgical (Dalla Costa, Minero et al. 2014) pain. These large animal scales all include ear position, appearance of the eyes, nostril dilation, and grinding of the teeth; two also include tension of the lips. In the horse, these pain-induced facial expressions were less pronounced during interaction with an observer, and in the cow, they tended to be displayed only in the absence of an observer. These results are an impressive example of cross-species translation in pain research (Chambers and Mogil 2015). However, such systems appear to perform best in acute pain models.

Quantitative Sensory Testing (QST) has been widely used in both man and laboratory animals as an indicator of both pain and analgesic efficacy. In a bovine experimental model of visceral pain, mechanical QST supported analgesic modulation of central pain, which was also detectable *via* behavioral assessment (Rialland, Otis et al. 2014). In osteoarthritic human patients, a significant relationship has been demonstrated between clinical pain ratings, and QST-assessed sensitization, specific to body region and pain modality (Arendt-Nielsen, Eskehave et al. 2014, Arendt-Nielsen, Nie et al. 2010). A clear association has also been observed between QST-assessed sensitization and pain-related behaviors in an experimental

canine model of osteoarthritis (Rialland, Otis et al. 2014). In both the latter and in the bovine model, biomarkers (lumbar spinal substance P and transthyretin in the dog, and cerebrospinal fluid transthyretin in the cow) also showed changes consistent with sensitization.

1.4.4.2 Pain assessment in veterinary patients

Clinical veterinary medicine typically makes use of subjective pain assessments by owners/caretakers and veterinarians/veterinary staff, as well as physiological measures (*e.g.*, blood pressure, heart/respiratory rate, body temperature, appetite, weight loss/gain). Despite recent professional guidelines recommending the use of standardized pain assessments (Epstein, Rodan et al. 2015), there is a dearth of validated and easy-to-use scales for animals (Paul-Murphy, Ludders et al. 2004). That being said, a handful of pain scales have been psychometrically validated (*i.e.*, tested for reliability and for evidence that they really measure what they are purported to) or partially validated for veterinary use. These range from uni-dimensional (*e.g.*, visual analog, simple descriptive scales) (Conzemius, Hill et al. 1997, Holton, Scott et al. 1998, Lascelles, Cripps et al. 1997) to composite scales incorporating both objective physiological and subjective criteria (Bussieres, Jacques et al. 2008, Firth and Haldane 1999). The Canine Brief Pain Inventory for assessment of bone cancer pain in dogs has been proposed as a parallel measure to that used in humans with the same condition (Brown, Boston et al. 2009). Quality-of-life assessments in companion animals may include categories on social functioning and sleep patterns (Zamprogno, Hansen et al. 2010), which are often affected in human pain; this suggests potential for comparative study applications. Significant barriers to more widespread adoption of these approaches in veterinary clinical practice are the time required to perform the assessment, and the difficulties associated with the environment in a busy veterinary clinic. Sounds, sights and odors from other veterinary patients may all influence the behaviors used in many of the pain scores. Despite these limitations, when efforts are made to conduct the assessments in as controlled conditions as possible, useful clinically relevant data on analgesic efficacy can be obtained (Reid, Nolan et al. 2007). Some of these problems can be overcome by using owner assessments. The pet-owner is often highly motivated and able to spend considerable time observing their companion animal, and recording this data, enabling relatively complex quality of life scores

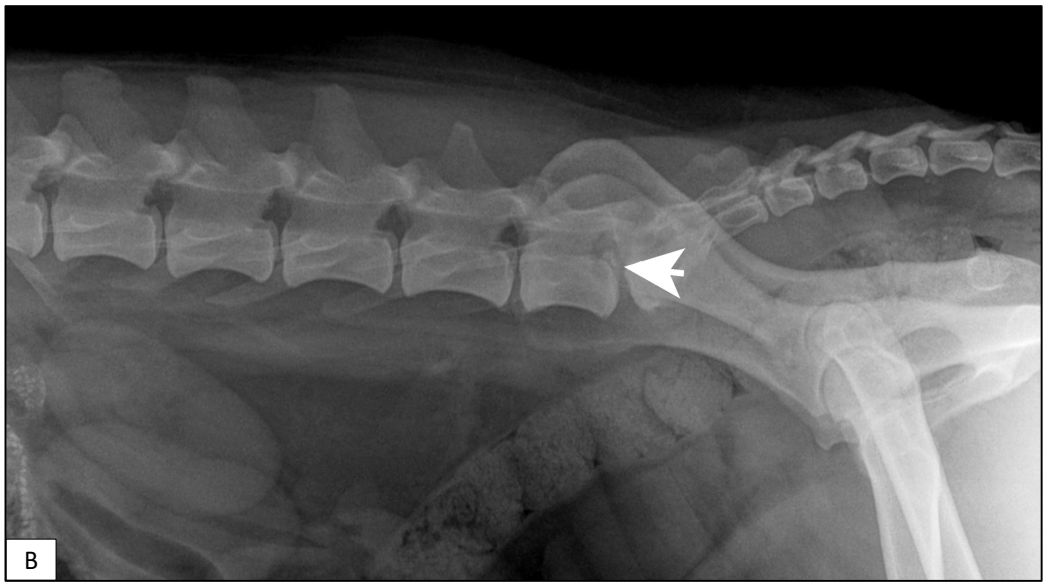
as well as evaluation of pain-related behavior (*e.g.*, (Rutherford, Wessmann et al. 2012, Wiseman-Orr, Scott et al. 2006).

Many tests used in experimental models are applicable to natural models. For instance, thermal and mechanical nociceptive threshold testing devices have been developed for a number of domestic animal species (Briley, Williams et al. 2014, Dixon, Robertson et al. 2002, Dixon, Taylor et al. 2007, Love, Murrell et al. 2011, Williams, Kirkpatrick et al. 2014), and functional measures (*e.g.*, activity monitoring, force plate analysis), operant tests, and neurological function tests (*e.g.*, fMRI, PET, EEG) are also available, and have similar potential and limitations as in experimental models. The Grimace Scales discussed above also have obvious potential for application in veterinary patients (Descovich, Wathan et al. 2017), as demonstrated by recent reports (Holden, Calvo et al. 2014).

An excellent example of a veterinary disease with potential for modeling human pain is **osteoarthritis (**or degenerative joint disease)**-associated pain** in pet animals. Both surgically induced and naturally-occurring models of osteoarthritis have been widely studied in dogs (McCoy 2015) and recently reviewed (Moreau, Pelletier et al. 2013). Osteoarthritis is commonly considered a mechanical problem in humans (and quadrupeds); pain results from nociceptive input when a damaged joint is subjected to mechanical stress during weight bearing or movement (Fig. 1.4.2) (Walsh 2016). However, the human experience of osteoarthritis pain often bears little relationship to the extent of joint damage as determined by traditional radiography (Hannan, Felson et al. 2000). Similarly, structural changes detected radiographically did not correlate well with expressed pain or functional impairment in dogs (Gordon, Conzemius et al. 2003) and cats (Guillot, Moreau et al. 2012). Factors such as synovitis, osteochondral pathology, and sensitization of both peripheral and central pain pathways each contribute to the quality and severity of osteoarthritis pain (Walsh 2016). Biomechanical, structural histological and macroscopic (Fig. 1.4.3) (McCoy 2015, Proffen, McElfresh et al. 2012), genomic, and molecular (Lorenz, Wenz et al. 2005) similarities were reported between human osteoarthritis (Little and Hunter 2013) and the dog model of osteoarthritis. Osteoarthritis in cats (Fig. 1.4.4) also appears to be very similar to the human condition (Freire, Meuten et al. 2014, Ryan, Lascelles et al. 2013). A structure-function (pain)

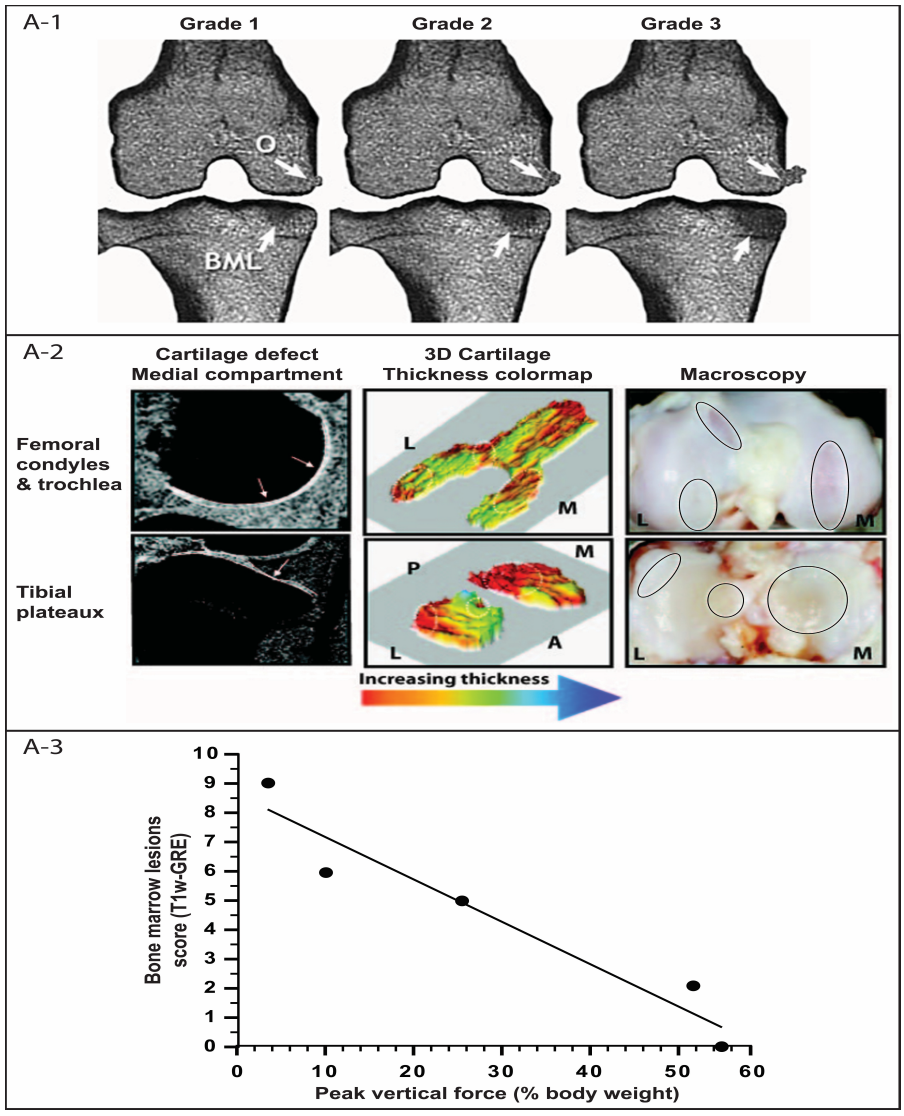
relationship has been translated from the dog model to the human osteoarthritis pain condition. Similar to reports in human patients (Torres, Dunlop et al. 2006, Zhang, Nevitt et al. 2011), pain in the canine osteoarthritis model, indirectly assessed by kinetics, evolved with bone marrow lesions (Fig. 1.4.3A-3), as well as progression of osteophytes, effusion/synovitis, and meniscal damage (Moreau, Pelletier et al. 2013). Unlike preclinical rodent models, the dog model of osteoarthritis offers translational positive predictive value with respect to therapeutic response. For example, structural and functional benefits of strontium ranelate have been translated from the dog experimental and natural models (Pelletier, Kapoor et al. 2012) to the human osteoarthritis patient (Reginster, Badurski et al. 2013). This is also true of doxycycline, local hyaluronan, bisphosphonates, diacerhein, licofelone and other NSAIDs (for references, see (Moreau, Pelletier et al. 2013)). This has recently been extended with the finding of functional benefits of anti-nerve growth factor in dogs (Lascelles, Knazovicky et al. 2015) and cats (Gruen, Thomson et al. 2016) with spontaneous osteoarthritis-associated pain, as has been shown in humans (Sanga, Katz et al. 2013).

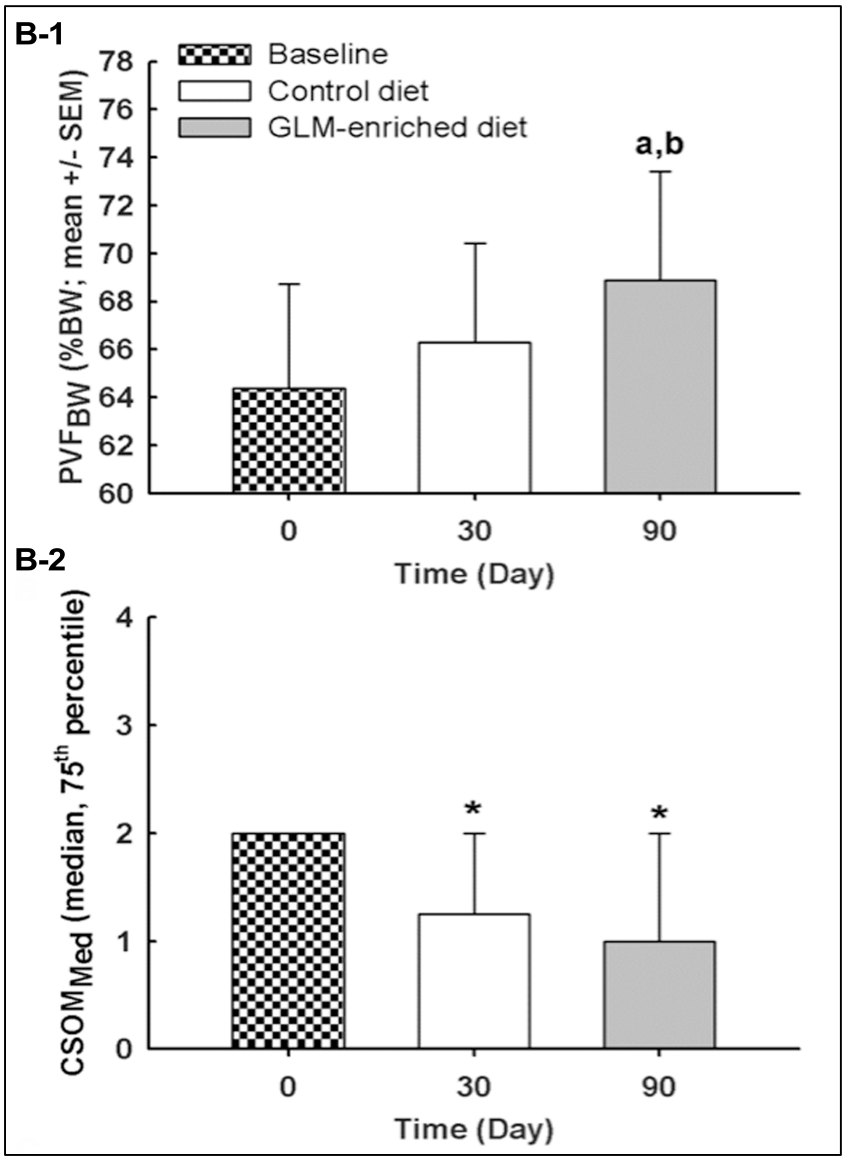
Figure 1.4.2: An 11-year-old Golden-Retriever dog with osteoarthritis of the left hip and a recent onset of episodic spontaneous pain.



Legend: (A) The dog displays body language consistent with stiffness and pain: head held in line with the back, tail hanging, crouched appearance of the hind limbs, stiff facial expression with tightening of the muscles of the forehead (between the eyes). (B) Radiographs of the lumbosacral spine revealed a lesion at L7-S1 consistent with extruded intervertebral disc material in the spinal canal (arrow). (© *CAP*douleur-2017)

Figure 1.4.3: Osteoarthritis in the dog as a translational model.





Legend: (A) Structural imaging of a canine osteoarthritic knee induced by experimental transection of the cruciate ligament demonstrating similarities with human osteoarthritis. Cruciate ligament rupture is the most common reason for orthopedic surgery in owned pet dogs in North America. (A-1) Schematic representation of grades 1–3 osteophytes (O) and bone marrow lesions (BML) as assessed on dorsal T1-weighed three-dimensional fast gradient recalled echo (T1w-GRE) magnetic resonance imaging (MRI) planes in canine stifles. (© URA/Arthro Vision-2017) (A-2) Correlation between MRI (left, arrows indicating cartilage defect); unfolded 3-D cartilage thickness reconstruction color map of

femoral condyles and trochlea (middle panel – top) and tibial plateaus (middle panel – bottom) 26 weeks after cranial cruciate ligament transection (CCLT) in experimental dogs. The red color represents areas with less cartilage; and macroscopic appearance of osteoarthritic cartilage 26 weeks post CCLT surgery. A, anterior; P, posterior; L, lateral; M, medial. Circles indicate lesions. (© URA/ArthroVision-2017) (A-3) Significant correlation ($r_s = –0.99$, $P < 0.001$) for the difference of BML scores on T1w-GRE during the remission phase (week 26 *minus* week four), with the concurrent difference in peak vertical force (PVF) measurement after CCLT in experimental dogs (Moreau, Pelletier et al. 2013). The increase in PVF (higher to the right, and suggestive of lower pain) measured during the remission phase correlated inversely with the score for BML (*i.e.*, the more BML, the more pain), osteophytes, joint effusion and focal changes of the articular cartilage (*data not shown*). The negative correlations mean an abrogated remission in the presence of MRI-scored severe chondral and subchondral lesions and joint effusion. (B) Evolution of primary clinical endpoints in naturally osteoarthritic dogs sequentially fed control and green-lipped mussel (GLM)-enriched diets. Graphs showing PVF (B-1) adjusted for body weight (%BW) and client-specific outcome measures (CSOM) median score (B-2) in response to dietary therapy in 23 privately-owned dogs with naturally-occurring osteoarthritis (Rialland, Bichot et al. 2012). (© GREPAQ-2017)

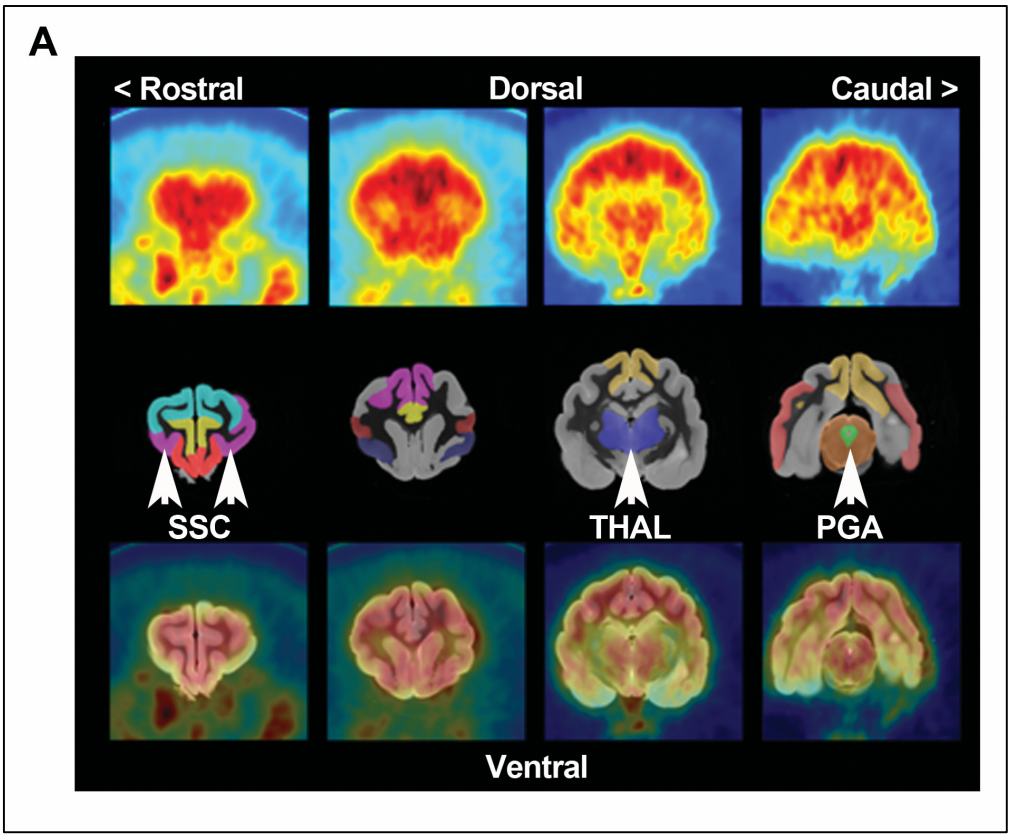
Figure 1.4.4: A colony of research cats with naturally-occurring osteoarthritis.

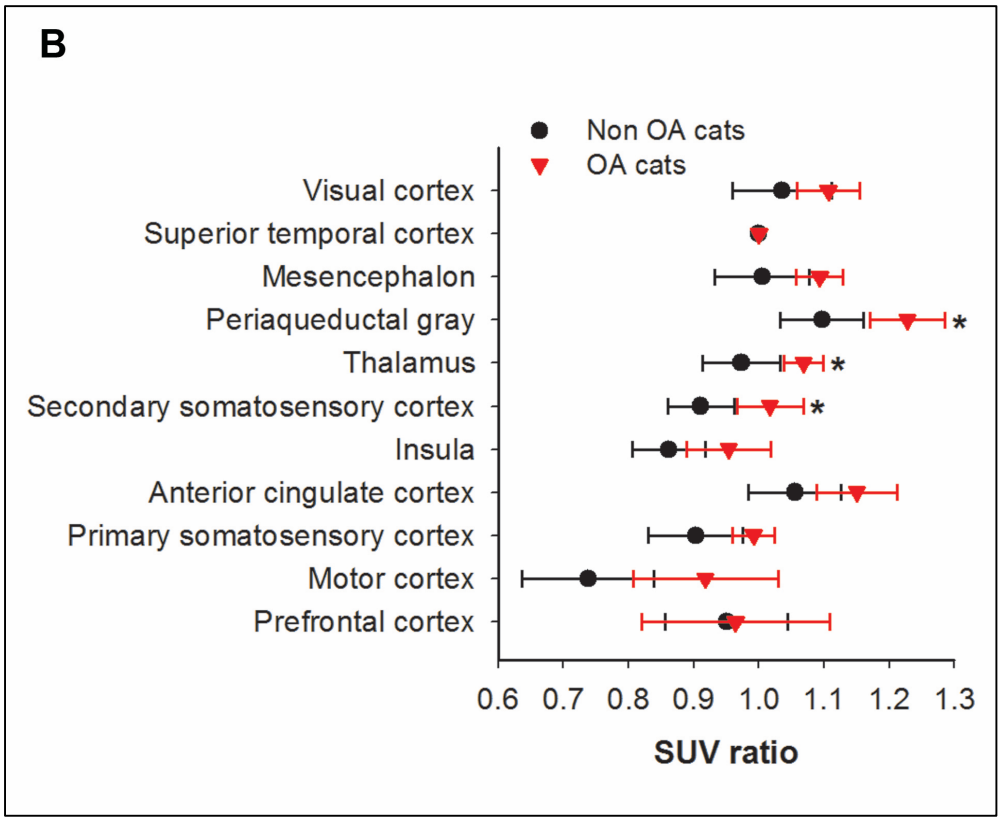
Legend: The cats were kept in groups, in an environment enriched with perches, hiding spots, beds, scratching posts, and window access, and multiple feeding, water, and litter sites, to facilitate social behavior and motor activity, which were monitored as welfare biomarkers. Collar-mounted telemetric activity monitors are indicated by arrows; they are used in the same way there are used in client-owned cats. Cats were screened for the presence of osteoarthritis and the absence of other significant disease, and then acclimated to the environment and testing procedures. Training (*e.g.*, to traverse a pressure-sensing walkway) was accomplished using positive reinforcement (treats, brushing, petting). These cats remained in the colony for 3 years, taking part in numerous studies. (© GREPAQ/URA-2017)

Recently, QST methodology showed clearly that dogs with naturally-occurring osteoarthritis have central sensitization (Knazovicky, Helgeson et al. 2016), manifesting as increased sensitivity to punctate mechanical, blunt mechanical, hot thermal and cold thermal

stimuli. This work demonstrates the fidelity with the human condition and paves the way for testing of antihyperalgesic drugs in this spontaneous model. Additionally, reversal of hyperalgesia following total joint replacement was found in dogs with hip osteoarthritis undergoing total hip replacement (Tomas, Marcellin-Little et al. 2014) as has been found in humans (Aranda-Villalobos, Fernández-de-las-Peñas et al. 2013). Sensitization with decreased mechanical QST thresholds has also been found in cats (Guillot, Moreau et al. 2013) with osteoarthritis, manifesting the presence of secondary punctate tactile allodynia. Central sensitization assessed by QST correlates with functional scores in osteoarthritic humans (Kuni, Wang et al. 2015), with joint pain scores in dogs with naturally-occurring osteoarthritis (Knazovicky, Helgeson et al. 2016) and with kinetics in the surgical model of canine osteoarthritis (Rialland, Otis et al. 2014). The facilitation of nociceptive temporal summation, observed in awake osteoarthritic cats, was positively correlated to the QST (von Frey anesthesiometer)-induced paw withdrawal threshold, compared to healthy cats (Guillot, Taylor et al. 2014). Temporal summation appears to be *N*-methyl-D-aspartate receptor-dependent in both animals and humans, is recognized as a manifestation of activity-dependent spinal wind-up, and is a well-recognized mechanism-based evaluation technique for musculoskeletal pain in humans (Arendt-Nielsen, Nie et al. 2010, Woolf 2011). Both central sensitization manifestations, *i.e.* secondary punctate tactile allodynia and enhanced mechanical temporal summation, reflect the sustained cerebral nociceptive inputs and increased activity of descending modulatory pathways (Fig. 1.4.5) observed with PET imaging in cats affected by natural osteoarthritis (Guillot, Chartrand et al. 2015). Interestingly, both QST manifestations of central sensitization in osteoarthritic cats were non-responsive to the NSAID meloxicam (Guillot, Moreau et al. 2013, Monteiro, Klinck et al. 2016), but as expected (Woolf 2011), mechanical temporal summation was responsive to tramadol, an atypical analgesic with serotonin/noradrenaline reuptake inhibitor effects capable of reinforcing descending inhibitory nociceptive pathways (Monteiro, Klinck et al. 2016). Mechanism-based pain diagnosis and treatment, such as this, provides an opportunity to improve translational research in osteoarthritis-associated chronic pain, such has been done recently with etoricoxib (Arendt-Nielsen, Egsgaard et al. 2016).

Figure 1.4.5: Functional imaging of feline cerebral areas altered in naturally-occurring osteoarthritis.





Legend: (A) Transverse sections of the osteoarthritis cat brain during positron emission tomography/magnetic resonance imaging techniques. Brain metabolism was significantly increased in osteoarthritic cats, in the secondary somatosensory cortex (SSC) as well as in the thalamus (THAL) and the periaqueductal grey matter (PAG) (Guillot, Chartrand et al. 2015). (B) Graph comparing brain region metabolic activity (expressed as means and standard deviations of standardized uptake values – SUV –) in osteoarthritic and non-osteoarthritic cats. Ratio = $SUV_{Region\ Of\ Interest}$ / $SUV_{Superior\ Temporal\ Cortex}$. * $P \leq 0.005$ (Guillot, Chartrand et al. 2015) (© GREPAQ-2017)

Dogs with osteoarthritis manifest measurable alterations in biomechanics, pain, and stress (Rialland, Bichot et al. 2012). Some composite scales for chronic pain (Bennett and Morton 2009, Brown, Boston et al. 2008, Hielm-Björkman, Rita et al. 2009, Walton, Cowderoy et al. 2013, Wiseman-Orr, Scott et al. 2006, Zamprogno, Hansen et al. 2010) and client-specific outcome measures (Lascelles, Hansen et al. 2007, Rialland, Bichot et al. 2012)

have been validated to varying degrees for use in dogs and cats. These caregiver-completed clinical metrology instruments capture primarily the ability of the pet to perform activities of daily living, but also other dimensions. Factor analysis of the two commonly used scales for canine osteoarthritis (Canine Brief Pain Inventory and the Liverpool Osteoarthritis in Dogs index) (Walton, Cowderoy et al. 2013) indicated that the latter is a 3-factor instrument (activity/exercise; stiffness/lameness; effect of weather) but for the Canine Brief Pain Inventory, all items loaded onto one factor. Recently, a clinical metrology instrument to assess sleep was found to show responsiveness validity (Knazovicky, Tomas et al. 2015), indicating that sleep patterns are disturbed in dogs with osteoarthritis just as in human chronic pain patients. Telemetered motor activity has been found to be a valid surrogate measure of distance moved in dogs (Hansen, Lascelles et al. 2007) and cats (Fig. 1.4.4) (Lascelles, Hansen et al. 2008), and further, found to be a sensitive method for assessing osteoarthritis (Guillot, Moreau et al. 2012, Moreau, Pelletier et al. 2013) and its treatment (Brown, Boston et al. 2010, Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2007, Rialland, Bichot et al. 2012), as it is related both to subjective pain assessment (Rialland, Bichot et al. 2012), and kinetics (Moreau, Pelletier et al. 2013). A 54-min increase in daily activity duration translates to significantly improved biomechanics for osteoarthritic dogs (Moreau, Pelletier et al. 2013), and this correlates very closely with the relationship between amount of exercise and lameness in dogs with osteoarthritis (Greene, Marcellin-Little et al. 2013). Kinetic gait analysis, which uses force plate variables, allows neuromuscular and skeletal appendicular disorders to be evaluated objectively both in animal (McLaughlin 2001, Moreau, Lussier et al. 2014, Schnabl and Bockstahler 2015) and human (Messier, Loeser et al. 1992) patients. This method, which is sensitive and repeatable under predefined standardized conditions, is considered the gold standard for assessing osteoarthritis in dogs (McLaughlin 2001, Moreau, Lussier et al. 2014, Moreau, Pelletier et al. 2013, Rialland, Bichot et al. 2012). However, it is influenced both by biomechanical and neurophysiological (particularly nociceptive sensitization) alterations, but does not distinguish between them. Other natural musculoskeletal disorders in animals present translational value for human conditions, such as tendon and ligament injuries, arthropathies and stress fractures (Innes and Clegg 2010).

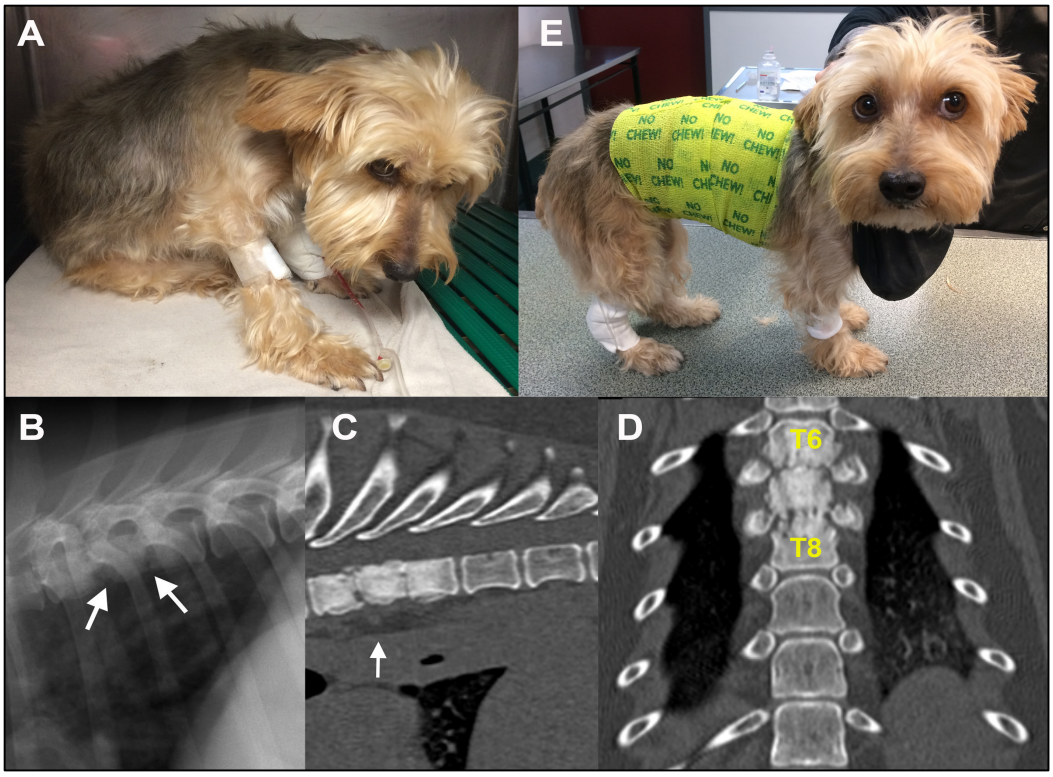
Another major field of interest for its translational value is **cancer** in pet animals (Alvarez 2014, Kol, Arzi et al. 2015, Paoloni and Khanna 2008, Paoloni, Webb et al. 2014). Although scientific evidence for some analgesics is available in canine oncology, to the best of our knowledge, no study has yet comprehensively evaluated the pain characteristics associated with cancer in dogs or cats; however, work in this area is underway (Health Canada – Experimental Study Certificate #183895; American Veterinary Medical Association Animal Health Studies Database #AAHSD000362) testing metrological properties of different pain outcome measures in cancer-bearing dogs. Some tumors, *e.g.* osteosarcoma, have long been recognized as being associated with severe pain, however this has yet to be studied systematically. Some early work has been performed testing the ability of the Canine Brief Pain Inventory to assess osteosarcoma-associated pain in dogs (Brown, Agnello et al. 2015). Objective force plates have been used to assess the analgesic effects of external beam radiation therapy in dogs (Weinstein, Payne et al. 2009), but unfortunately the study was severely underpowered. Other work has been performed investigating aspects of the neurobiology of osteosarcoma pain in dogs (Shor, Fadl-Alla et al. 2015).

Other potential applications, described in Figure 1.4.1, highlight **neuropathic pain** conditions. The prevalence of such conditions in companion animals is unclear due to difficulties in assessment and diagnosis. Their translational value is therefore dependent on the establishment of valid and reliable outcome measures. They may be congenital (such as Chiari-like malformation and syringomyelia in Cavalier King Charles Spaniels) or acquired, may affect any body system, and can be associated with traumatic, surgical, infectious, degenerative (such as intervertebral disc disease, corneal ulceration, osteoarthritis), metabolic (diabetes), neoplastic, or toxic (vincristine, cisplatin) processes.

For example, intervertebral disc disease can be associated with radiculopathy; it occurs more commonly in the thoraco-lumbar than in the cervical region, with the latter being more painful (Fig. 1.4.6) than paretic compared to the former (Brisson 2010). Degenerative lumbosacral stenosis (*cauda equina* syndrome), resulting from compression of the sciatic, pudendal, pelvic and caudal (L7-Cd5) nerves, is quite common in dogs. It is associated with positional pain, hyperesthesia, paresthesia and self-mutilation of the lumbo-sacral area and

pelvic limbs (Meij and Bergknut 2010). Sacro-caudal luxations (tail-pulling injury in the cat), pelvic fractures, phlegmons induced by bites at the tail base, and spondylitis cause articular nociceptive (inflammatory) and deafferentation (neurogenic) pain. Translational value in ocular pain has been demonstrated for intraocular neoplasia, corneal epithelial and stromal disease, uveitis, and glaucoma (He, Li et al. 2013, Zeiss 2013).

Figure 1.4.6: A 7-year-old Yorkshire Terrier which had been treated for pyometra one month beforehand developed neck pain and pain associated with the thoracic spine.



Legend: (A) The dog's posture and facial expression are consistent with neck pain: the head and neck are held stiffly, in an extended and slightly lowered position, and the facial expression is anxious (ears drawn back and down, wide open "moon" eyes with sclera visible). Due to the long hair coat, it is difficult to evaluate other features (such as wrinkling of the skin), but the face appears generally tense. (B) A lateral radiograph of the thoracic spine showing lysis of the

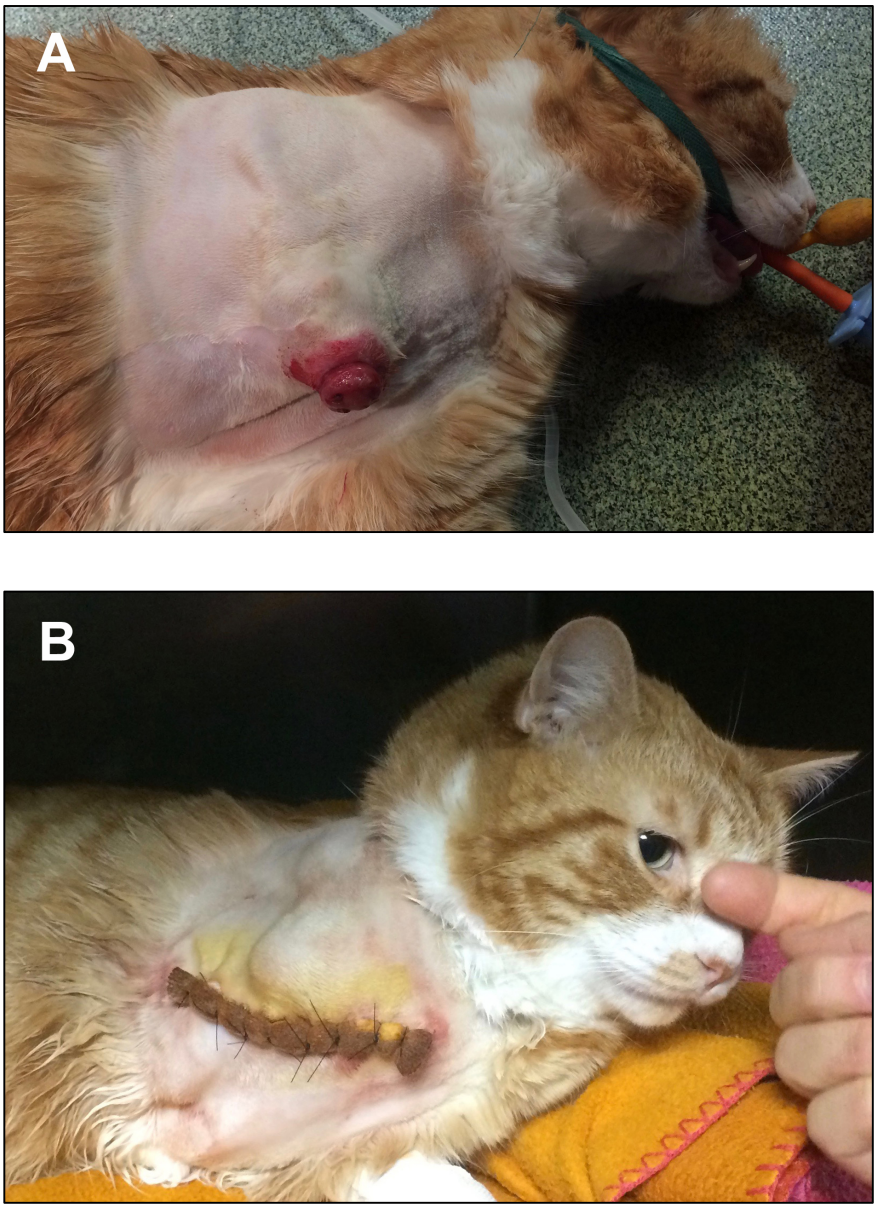
vertebral endplates at T6-8 (arrows). (C) CT-scan imaging (lateral) of the affected spinal segments, consistent with discospondylitis (arrow). (D) CT-scan imaging (ventrodorsal) of the affected spinal segments consistent with discospondylitis (T6 and T8 labelled). (E) The same dog demonstrating a non-painful posture following antibiotic and analgesic (gabapentin, and constant rate infusion of ketamine, lidocaine, and fentanyl) therapy. (© *CAP*douleur-2017)

In veterinary practice, the incidence of persistent postoperative chronic pain is unknown and probably underestimated. Surgical procedures can transect terminal branches, generating neuromas, or cause stretching (dissection, tissue deformities). The suture may accidentally ligate nerve fibers. Finally, the healing of extensive wounds (*e.g.*, following surgical removal of a mammary chain or fibrosarcoma) may cause entrapment of nerves within a fibrotic reaction. There are two types of pain after limb amputation:

- Stump pain (peripheral origin) secondary to various pathologies including cutaneous lesions, vascular injuries, neuromas, *etc.*
- Phantom limb pain for which a central origin is suspected.

Either phenomenon may arise in animals (Fig. 1.4.7). Onychectomy (declawing) is a source of intense ethical debate around the world, due in part to the suspicion that neuropathic pain may be generated by the procedure where analgesia/anesthesia is inadequate.

Figure 1.4.7: A cat demonstrating self-mutilation post-amputation of the right front limb.



Legend: Spontaneous pain secondary to neuroma formation or phantom limb pain was diagnosed. (A) Wound site during preparation for surgical wound repair. (B) Wound site one day after this surgery. Self-mutilation resolved following surgery and analgesic treatment (methadone, ketamine, gabapentin and meloxicam). (© *CAP*douleur-2017)

Feline diabetic neuropathy is associated with proprioceptive deficits, muscular deficits, progressive paresis, and sometimes a characteristic plantigrade stance and locomotion. *Diabetes mellitus* is a common endocrinopathy in cats with a recently described incidence rate of 11.6 cases per 10,000 cat-years at risk (out of a total of 1,229,699) (Öhlund, Fall et al. 2015). Male cats have twice as high an incidence rate as females. Pain, very common in humans, is not often described in the cat, probably due to lack of detection, but perhaps also because of a shorter lifespan, since a correlation exists between the duration of diabetic disease and the occurrence of neuropathic pain.

Stroke also occurs in dogs and cats (Platt and Garosi 2003), but is difficult to distinguish from vestibular syndrome, which is much more common and has similar clinical signs such as a head tilt, walking in circles, rolling to one side, ataxia, *etc.* The prevalence of associated pain is not known, but should be considered, particularly where animals have severe behavioral sequelae. An assessment of mood disorders (anxiety, depression) must be part of a quality of life evaluation after a vascular accident. Migraine-like symptoms have also been recently described in a dog (Plessas, Volk et al. 2013).

Inflammatory bowel disease is commonly encountered in dogs and cats, as is feline interstitial cystitis (Fig. 1.4.1), and pathogenesis involves neurogenic inflammation. Skin disease and injuries are very common reasons for consultation in veterinary practice. Self-injury linked to intense pruritus produces pain *via* inflammatory or neuropathic mechanisms. Self-directed oral behaviors (sucking, licking, chewing) may result from sensory abnormalities caused by neuropathy; however, primary dermatologic disease (*e.g.*, allergic dermatitis), orthopedic, or other medical disease, or behavioral causes (*e.g.*, anxiety, compulsive behavior) are other possible causes (Frank 2014). Neuropathic pruritus is observed with central nervous system lesions, such as syringomyelia. The Cavalier King Charles Spaniel, for instance, is prone to a Chiari-like malformation, which is often accompanied by syringomyelia (Cappello and Rusbridge 2007). The clinical picture is dominated by neuropathological signs (paresis, ataxia), and signs of pain (neck guarding posture, scratching at the head and neck with/without making contact with the skin, as well as screaming spontaneously, during movement, or when the area is touched for example by the collar) (Fig. 1.4.8). Feline hyperesthesia syndrome may

be associated with a sensory neuropathy such as neurogenic pruritus. It is characterized by episodes of agitation, vocalization, focal spasms of the epaxial muscles and twitching of the skin on the dorsum, staring at the tail, running away, and intense biting/licking of the back, tail, and sometimes the pelvic limbs. It may be associated with redirected aggression to humans or animals (Ciribassi 2009).

Figure 1.4.8: A Cavalier King Charles Spaniel affected by a Chiari-like malformation accompanied by syringomyelia.

Legend: The dog demonstrates a hunched posture with a stiffly held head and neck, knuckling of the right front paw (proprioceptive deficit), and a base wide, crouched stance of the rear limbs, associated with paresis or pain. The tail is down, associated either with paresis or with anxiety/fear, and the eyes are wide (anxiety). (© *CAP*douleur-2017)

1.4.4.3 Practical considerations and potential barriers to the use of naturally-occurring models in animals

Incorporation of the study of spontaneous painful conditions in animals after preclinical research and before human clinical trials would be hoped to complement laboratory animal studies and to reduce failures in clinical trials. Although the study of natural models has considerable potential as an adjunct to the current drug development process, there are some significant considerations and practical issues that need to be overcome. With respect to species choice, companion animals, in addition to sharing the owners' environment and to receiving much individual attention from owners, are more amenable to novel drug testing in the course of treatment of spontaneously-occurring disease, than are livestock. Commercially owned food-producing animals, in particular, are unlikely to be good candidates for such testing, primarily due to the concerns over drug residues in meat or milk products. For instance, the pig has demonstrable value in experimental (post-surgical (Gigliuto, De Gregori et al. 2014)), locomotor (Nalon, Conte et al. 2013) and particularly transgenic models (Roth and Tuggle 2015), and spontaneously develops some painful diseases like those in humans (*e.g.*, osteochondrosis (McCoy 2015), chronic and potentially painful gastrointestinal symptoms secondary to early weaning stress (Pohl, Medland et al. 2015)). However, commercially owned swine tend to be of low monetary value and to receive little individual veterinary attention. They are also generally raised under intensive (unvaried, constrained) conditions (*i.e.*, indoors, with limited space and environmental enrichment, with some exceptions for instance in organic farming), and (with the exception of breeding animals) only kept for a few months before being slaughtered for meat. Dairy cows, on the other hand, tend to be kept over the longer term, in more varied (indoor/outdoor) environments, and can have a high individual value (and hence receive state of the art care); non pharmaceutical pain research could potentially incorporate such animals. Other livestock such as the horse, wool-producing sheep and goats, and possibly the pet pig, might be considered as candidates for drug development studies (see Figure 1.4.1). These usually live in relatively complex environments, and as companion or high monetary value animals, they can receive high quality medical care. However, they present some challenges such as not sharing the human environment and having the potential to enter the food chain eventually, or being too

uncommon to be a practical resource (pet pig). It therefore seems advisable for initial development of the use of naturally-occurring painful disease models in veterinary patients, to begin with the dog and cat.

Some painful conditions in companion animals are ready or close to ready for implementation as natural models of human pain (*e.g.*, osteoarthritis, intervertebral disk disease, various cancers). Others may require varying degrees of development with respect to understanding of disease pathophysiology and establishment and validation of outcome measures specific to the condition of interest (*e.g.*, inflammatory bowel disease pain). As is the case in human patients, invasive procedures and tissue harvesting may not be possible, unless they are to the benefit of the (animal) patient (*e.g.*, diagnostic biopsy, medically indicated surgery) or if collections are performed at necropsy. One important consideration is that euthanasia of pets is commonly performed, and these pets can be phenotyped prior to euthanasia using specific evaluation and/or the medical records. In terms of using pets in proof of concept therapeutic studies, inter-animal and environmental variability would also likely contribute to difficulty in detecting clear patterns of disease and therapeutic responsiveness, necessitating larger sample sizes. As is the case when recruiting human patients for clinical trials, accurate information on the incidence of the condition in the companion animal population is required. For many veterinary diseases, incidence has not been reported; some examples of conditions for which either incidence or prevalence data is available are listed in Table 1.4.1. Databases enabling such estimates to be made are now becoming more widely available and have been applied to estimating incidence of a number of relevant conditions (Bergknut, Egenvall et al. 2012, Meij and Bergknut 2010, Sanchis-Mora, Pelligand et al. 2016). Other challenges to the use of veterinary patients as pain models include subject recruitment (Gruen, Jiamachello et al. 2014) and retention, and compliance with study protocols. Only very recently have the potential barriers to subject recruitment been investigated (Gruen, Jiamachello et al. 2014), and they appear to be similar to the barriers present in human medicine trials, and especially pediatric studies. A potential difficulty is that a large proportion of initial presentations of animal patients are undertaken in private veterinary practices with relatively small case loads, and primary care veterinarians may be unaware of open veterinary clinical trials (Gruen, Griffith et al. 2016). Nonetheless, this

problem might be circumvented by working with large networks of veterinary practices; for instance, the Mars company has acquired VCA Animal Hospitals, which, in addition to the pet hospitals it already owns, will make Mars the owner of 1,840 veterinary practices in North America. The American Veterinary Medical Association has also recently launched an online Animal Health Studies Database, with the goal of connecting researchers with primary care veterinarians. The number of large referral practices is increasing world-wide (*e.g.*, the American Animal Hospital Association accredits about 350 such practices in the USA and Canada). The American Veterinary Medical Association, which accredits veterinary schools, encourages academic institutions to conduct veterinary medical research, promotes the application of knowledge gained from spontaneously-occurring disease in animals to therapies in human and animal medicine, and considers translation of medical discoveries in both directions (human-animal and animal-human) fundamental to the veterinary profession as well as to biomedical research (Baneux, Martin et al. 2014). Veterinary schools have always represented a source for recruitment of a study population. Although the logistics of the process will be far more complex than is the use of rodent models within a research facility, the clinical trials of veterinary products, including analgesics, illustrate that this is achievable, at least for common conditions (*e.g.*, post-surgical pain (Taylor, Kirby et al. 2010, Wagner, Worland et al. 2008)). In addition, there exist contract research organizations specializing in veterinary clinical trials (*e.g.*, VetPharma in the USA and Europe). A recent review has also demonstrated that a relatively high percentage of referral practice veterinarians surveyed (approximately 50% of the group surveyed) were involved in clinical research (Fordyce and Mullan 2017). This survey also demonstrates that, at least in some areas of research, support at an Institutional level is available for this type of work in veterinary schools and referral practices. The Comparative Oncology Trials Consortium is an active network of twenty academic comparative oncology centers, centrally managed by the National Institutes of Health-National Cancer Institute-Center for Cancer Research's Comparative Oncology Program. It designs and executes clinical trials to assess novel therapies in dogs with cancer, demonstrating how collaborative research can function effectively. The goal of this effort is to answer biological questions, informing the development path of these agents for future use in human cancer patients. Trials conducted by the Comparative Oncology Trials Consortium are pharmacokinetically and pharmacodynamically rich with the products of this work integrated

directly into the design of current human Phase I and II clinical trials. The trials are carried out at member institutions, of which there are currently 22 sites. A recent development is the Clinical and Translational Science Award One Health Alliance (https://ctsaonehealthalliance.org) supporting a national (USA) network of medical research in diseases shared by humans and animals.

Table 1.4.1: Examples of naturally-occurring painful conditions of animals and their reported prevalence or incidence.

Condition	Prevalence	Incidence	Pain Severity
Canine osteoarthritis/ degenerative joint disease	Estimated to affect 80% of dogs > 8 years old (Johnston 1997)	Unknown	Mild to severe
Feline osteoarthritis/ degenerative joint disease	Estimated to affect 60-93% of adult cats of which an estimated 40% have obvious signs of impairment due to musculoskeletal pain (Lascelles, personal communication)	Unknown	Mild to severe
Canine intervertebral disc (IVD) degeneration-related diseases DYAR = Dog-years at-risk	IVD herniation occurs in 2% of pet dogs, and in 19-24% of Dachshunds (Brisson 2010)	Swedish pet insurance records for dogs < 12 years old (total of 2,772,423 DYAR): Overall: 27.8 cases/10 000 DYAR Breed-specific incidence ranged from 2.0 cases/10,000 DYAR for the least affected to 237.1 cases/10,000 DYAR for the most affected breed (the Miniature Dachshund; having a total of 15,433 DYAR) (Bergknut, Egenvall et al. 2012)	Mild to severe

Table 1.4.1 (continued)

Condition	Prevalence	Incidence	Pain Severity
Canine cervical spondylomyelopathy and cervical IVD herniation		Swedish pet insurance records for dogs ≤ 12 years old (total of 2,772,423 DYAR): Overall: 3.0 cases/10 000 DYAR Breed-specific incidence ranged from 0.0 cases/10,000 DYAR to 58.6 cases/10,000 DYAR for the most affected breed (the Doberman Pinscher; having a total of 12,411 DYAR) (Bergknut, Egenvall et al. 2012)	Severe
Canine thoracolumbar IVD herniation		Swedish pet insurance records for dogs ≤ 12 years old (total of 2,772,423 DYAR): Overall: 3.7 cases/10,000 DYAR Breed-specific incidence ranged from 0.0 cases/10,000 DYAR to 41.0 cases/10,000 DYAR for the most affected breed (the Miniature Dachshund; having 15,433 DYAR) (Bergknut, Egenvall et al. 2012)	Severe

Table 1.4.1 (continued)

Condition	Prevalence	Incidence	Pain Severity
Canine lumbosacral IVD herniation and degenerative lumbosacral stenosis (DLSS)		Swedish pet insurance records for dogs ≤ 12 years old (total of 2,772,423 DYAR): Overall: 5.6 cases/10,000 DYAR Breed-specific incidence ranged from 0.0 cases/10,000 DYAR to 27.9 cases/10,000 DYAR for the most affected breed (the German Shepherd Dog; having 188,356 DYAR) (Bergknut, Egenvall et al. 2012)	Mild to severe
Canine DLSS		Swedish pet insurance records for commonly-affected dog breeds: 33.7 cases/10 000 DYAR for the German Shepherd Dog 21.7 for the Boxer 18.0 for the Rottweiler 17.5 for the Doberman Pinscher 8.8 for the Labrador Retriever (Meij and Bergknut 2010)	Mild to severe

Table 1.4.1 (continued)

Condition	Prevalence	Incidence	Pain Severity
Canine Chiari-like malformation/ syringomyelia	0.05% of dogs attending primary care veterinary practices in England (Sanchis-Mora, Pelligand et al. 2016) 1.6-1.7% of Cavalier King Charles Spaniels attending primary care veterinary practices in England (Sanchis-Mora, Pelligand et al. 2016, Summers, O'Neill et al. 2015)	Unknown	Mild to severe

Assessment of novel compounds in companion animals also raises ethical and legal issues, as does all clinical research. Ethical oversight is already in place in many institutions in the UK (Fordyce and Mullan 2017), the European Union, Austral-Asia, and North, Central and South America; however, it may be lacking in private primary care and referral veterinary practices outside of institutional collaboration (Baneux, Martin et al. 2014). Legal constraints can increase the complexity of the process, but are essential if public confidence in the integrity of the veterinary profession is to be retained. However, using legislation primarily designed to regulate use of laboratory animals may not be the most efficient way of achieving this oversight. Enrolling companion animals in clinical research raises a series of specific ethical issues that need addressing (Page, Baneux et al. 2016). For example, many potential subjects may already be receiving an effective therapy, and withdrawing this in order to evaluate a potentially more effective agent must be undertaken with appropriate safeguards for the animal's welfare. Additionally, any compound tested in companion animals would need to

have undergone safety testing in that species – indeed, this is currently demanded by Clinical Research Ethical Boards or Institutional Animal Care and Use Committees that oversee veterinary clinical research in academic institutions in North America.

An additional practical issue relates to dosing and dose regimens in companion animal species. Interspecies allometric scaling for dose conversion between species is already performed between laboratory animals and humans, and between these and other animals. This method uses body surface area and is premised on the fact that larger animals have lower metabolic rates and therefore require proportionately lower drug dosages (Nair and Jacob 2016). Additional work will be needed to determine pharmacokinetic and pharmacodynamics of the compound in these new target species, and sufficient compound synthesized to allow treatment of animals of significantly larger body weight than laboratory rodents. Since administration of the compound will often be by the owner, issues of compliance arise; however, since owners are usually very highly motivated this may be no worse, or even better, than in clinical trials in people (Adams, Campbell et al. 2005, Kardas 2002).

1.4.5 Conclusion

Veterinary patients benefit from our understanding of pain management in man; they also have the potential to contribute to our knowledge of human pain (Flecknell 2008, Quessy 2010). The study of natural animal models of pain could provide complementary information to that obtained from experimental models and address some of their limitations. It could also benefit veterinary patients themselves. Similar to the use of canine oncology patient translational models, controlled, effective and ethical implementation of natural animal models of pain would require substantial resources.

Interestingly, the development and use of validated methods of pain assessment is receiving increasing attention in the veterinary community, making this an excellent time for collaboration to share existing, and to validate new, measures. Multi-directional transfer of information between pain researchers, basic or applied scientists, physicians, veterinarians, specialists or general practitioners, will likely contribute to the best possible modeling of human patients' pain experience.

1.4.6 Acknowledgements

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1.5 Research Hypothesis and Objectives

1.5.1 Background (summary)

Pain is a complex biological phenomenon. It is difficult to predict because its experience varies between individuals with the same pathology; quantifying it is challenging in part because it is composed of sensory and affective aspects, and its expression differs between species, and even between and within individuals (*e.g.*, due to context). There is no perfect outcome measure; existing objective measures each assess different aspects of pain. Pain scales are commonly used in humans and, while inherently subjective, afford a relative objectivity. They require no specialized equipment and permit comparisons within and sometimes between individuals; however, they require validation prior to clinical use. Radiographic OA is common in cats and the evidence supports that OA causes clinical disease. However, cats are particularly challenging to assess for pain, and OA signs, in particular, seem to be difficult to detect in this species. At the beginning of this project, there were no pain scales validated for feline OA clinical diagnosis and disease monitoring.

1.5.2 Research hypothesis

It was hypothesized that feline OA pain and associated functional impairment would be measurable *via* standardized assessments using specific signs. Based on findings previously reported in the literature, and the research group's knowledge of cat behavior, and animal pain assessment methods, it was anticipated that such signs could involve mobility and activity, social, play, exploratory and self-maintenance (*e.g.*, ingestive, eliminative, and grooming) behaviors, and physical condition (*e.g.*, BCS, coat and claw condition, posture, gait, and pain or other palpable abnormalities upon examination of the joints). In addition, signs of OA pain were expected to be detectable both in the cat's home environment, and in the context of a veterinary examination.

1.5.3 Study objectives

The overarching goal of this project was to develop and validate two feline OA pain scales, one for cat owners, to aid in the assessment of behavior in the home for signs of OA pain, and one for use by veterinarians, to aid in OA pain detection and measurement during the physical examination. The following were specific objectives of this project:

1) Review of veterinary criteria for diagnosis, and determination of owner-observed signs in the home *via* a survey, for cats with a diagnosis of OA;
2) Item generation for each scale, based on review of the literature, expert opinion, and, in the case of the owner pain scale, interviews of owners of cats with a diagnosis of OA;
3) Development of response options and format, including total score calculation method, for each scale;
4) Consultation of experts to evaluate the content of both scales for comprehensiveness, appropriateness of content, and comprehensibility (content validation);
5) Consultation of cat owners (owner pain scale) and third-year veterinary students (veterinary pain scale) to evaluate end-user perceptions of clarity, comprehensiveness, and appropriateness of content of each scale (face validity; acceptability);
6) Comparison of ratings with each scale for a) the same user over time (intra-rater reliability), and b) different users concurrently (inter-rater reliability);
7) Evaluation of scores on both scales to examine relationships between scale items and subscales, and between scale items and scale total (internal consistency reliability);
8) Comparison of OA and non-OA cats using each pain scale to evaluate distinction of OA *vs.* non-OA cats (evidence of validity based on response processes; construct validity);
9) Comparison of pain scale scores with other measures of OA pain (evidence of validity based on relations to other variables; construct validity, convergent validity);
10) Comparison of pain scale scores before and after analgesic treatment in OA cats (evidence of validity based on response processes; construct validity, hypothesis testing).

2. PUBLICATIONS

A similar process was used to develop the content for both feline OA pain scales. This included a review of the literature for reported signs of OA observable by owners in the home and detectable upon physical examination, and expert opinion in feline behavior and pain. In the case of the owner pain scale, additional information was sought *via* a survey of owners of cats with a diagnosis of osteoarthritis (objective 1). The survey and its results are described in the first article that follows, "*Owner-perceived signs and veterinary diagnosis in 50 cases of feline osteoarthritis*" (Klinck MP, Frank D, Guillot M, Troncy E), published in the *Canadian Veterinary Journal* (2012, 53(11): 1181-1186). Author contributions to this article were as follows: Mary Klinck developed and participated in revising all the study materials, performed all study procedures, conducted the descriptive analysis of the data, wrote the initial version of the article manuscript and coordinated revisions to it. Diane Frank participated in the development of and revisions to the phone survey instrument, and participated in revising the article manuscript. Martin Guillot participated in the data analysis and in revising the article manuscript. Eric Troncy initiated the study and obtained and managed the funding for it, and participated in the development of and revisions to the study instruments, data analysis, and writing of and revisions to the article manuscript, in addition to supervising the work overall.

Following preliminary content development (objectives 2 and 3), both scales underwent a standard content validation phase *via* expert review (objective 4), and both were preliminarily evaluated for their validity and reliability *via* a pilot laboratory study in a colony of laboratory cats (objectives 6, 7, and 8). On the basis of the latter pilot study, it was determined that all further evaluation of the owner pain scale would require its application in client-owned cats. The second article, "*Development and preliminary validity and reliability of the Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner, MI-CAT(C), via a randomised clinical trial"* (Klinck MP, Gruen ME, del Castillo JRE, Guillot M, Thomson AE, Heit M, Lascelles BDX, Troncy E), describes validity and reliability testing of this scale in a placebo-controlled, double-blind clinical trial involving a group of client-owned cats with OA (objectives 5, 6, 7, 9, and 10). This article has been submitted to *Applied Animal Behaviour Science*. Author contributions to this article were as follows: Mary Klinck

developed the initial content and format for the MI-CAT(C), and developed the content validation questionnaire, contributed to the study design, performed some of the study procedures (for the content validation and pilot study phases), participated in the analysis of the data (content validation, pilot study, and clinical trial phases), and wrote, and coordinated the revisions to, the article manuscript. Margaret Gruen participated in the conception and design of, and performed study procedures for, the clinical trial, as well as participated in the revisions to the article manuscript. Jérôme del Castillo contributed to the data analysis for the clinical trial, and participated in the revisions to the article manuscript. Martin Guillot participated in the conception and design of, and in performing procedures for, the pilot study, contributed to the data analysis, and participated in the revisions to the article manuscript. Andrea Thomson contributed to the conception and design of, and performed study procedures for, the clinical trial, and participated in the revisions to the article manuscript. Mark Heit contributed to the conception and design of the clinical trial and participated in the revisions to the article manuscript. Duncan Lascelles participated in content validation of the MI-CAT(C), contributed to the conception and design of the clinical trial, obtained and managed the funding for the latter, participated in the revisions to the article manuscript, and contributed to the overall supervision of the work. Eric Troncy participated in developing the study materials (MI-CAT(C) and content validation questionnaire), contributed to the conception and design of, as well as to data analysis for, all experiments, participated in the revisions to the article manuscript, and contributed to the overall supervision of the work.

Following its development and preliminary validation in the pilot study, the veterinary pain scale underwent some refinements and was re-evaluated for reliability and validity *via* a larger study of laboratory cats with and without naturally-occurring OA; it was also evaluated for face validity *via* a review by third-year veterinary students (objectives 5, 6, 7, 8, and 9). This process and the study results are described in the third article, "*Preliminary validation and reliability testing of the Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians, in a colony of laboratory cats*" (Klinck MP, Rialland P, Guillot M, Moreau M, Frank D, Troncy E), published in *Animals (Basel)* (2015, 5(4): 1252-1267). The veterinary scale was unfortunately unable to distinguish OA from non-OA cats in either of these two studies. Author contributions to this article were as follows: Mary Klinck developed the

preliminary content and format for the MI-CAT(V), developed the questionnaire for and coordinated the content validation *via* expert review, participated in performing the experiments (pilot study and main study), contributed to the analysis of the data, wrote the article manuscript and coordinated the revisions to it. Pascale Rialland contributed to the conception and design of the experiments, participated in performing the pilot and main study procedures, contributed to the data analysis, and participated in the revisions to the article manuscript. Martin Guillot contributed to the conception and design of the experiments, participated in performing the pilot and main study procedures, contributed to the data analysis, and participated in the revisions to the article manuscript. Maxim Moreau participated in performing the pilot and main study procedures, and in the revisions to the article manuscript. Diane Frank participated in the development of the content and format for the MI-CAT(V), and participated in the revisions to the article manuscript. Eric Troncy initiated the study and obtained and managed the funding for it, and participated in the development of the content and format for the MI-CAT(V), the conception and design of the experiments, the performance of study procedures, and the revisions to the manuscript, in addition to supervising the work as a whole.

Following the above study, major revisions to the scale were made, on the basis of a video analysis, in an attempt to improve sensitivity to OA. Subsequently, the veterinary scale was evaluated for validity and reliability and underwent further revisions, all in the context of a series of therapeutic trials, again in colonies of laboratory cats with and without naturally-occurring OA (objectives 6, 7, 8, 9, and 10). This is described in the fourth article, "*Refinement of the Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians: detection of naturally occurring osteoarthritis in laboratory cats"* (Klinck MP, Monteiro BP, Lussier B, Guillot M, Moreau M, Otis C, Steagall PVM, Frank D, Martel-Pelletier J, Pelletier J-P, del Castillo JRE, Troncy E), published in the *Journal of Feline Medicine and Surgery* (2017, doi: 10.1177/1098612X17730172). Author contributions were as follows: Mary Klinck contributed to the conception and design of the studies, participated in performing all the studies, developed the questionnaires for the video analysis and the surgeon's orthopedic evaluation, performed the revisions to the MI-CAT(V), contributed to the data analysis, wrote the article manuscript, and coordinated the revisions to the latter. Beatriz Monteiro

participated in performing the studies (Phases II and III), contributed to the revisions to the MI-CAT(V), and participated in the article manuscript revisions. Bertrand Lussier participated in developing the surgeon's orthopedic assessment questionnaire, performed part of the video analysis and Phase I studies, and participated in revising the article manuscript. Martin Guillot contributed to the conception and design of the studies, performed study procedures (Phases I-III), participated in the data analysis, and participated in the revisions to the article manuscript. Maxim Moreau and Colombe Otis each participated in the study procedures (Phases I-III), contributed to the data analysis, and participated in the revisions to the article manuscript. Paulo Steagall contributed to the conception and design of the studies, and participated in the revisions to the article manuscript. Diane Frank participated in the study procedures (video analysis) and in the revisions to the article manuscript. Johanne Martel-Pelletier and Jean-Pierre Pelletier contributed to the conception and design of the experiments, and participated in the revisions to the article manuscript. Jérôme del Castillo contributed to the data analysis and participated in the revisions to the article manuscript. Eric Troncy initiated the study and obtained and managed the funding for it, contributed to the conception and design of all studies, participated in the development of and revisions to all study materials, participated in performing the studies, contributed to the revisions to the manuscript, and oversaw the work as a whole.

2.1 Owner-perceived signs and veterinary diagnosis in 50 cases of feline osteoarthritis[10]

Klinck MP[11]*, Frank D[12], Guillot M[2], Troncy E[2]

2.1.1 Abstract

Veterinarians contacted to identify cats diagnosed with osteoarthritis (OA) provided information on signalment, method of diagnosis, treatment and concurrent disease. Owners of 50 cats were interviewed to collect information on specific OA signs observed in the home, relating to mobility, self-maintenance, social and exploratory behavior, and activity and habits at diagnosis and after treatment. Mean age at diagnosis was 12 y; concurrent diseases were common (44%). Owner-reported abnormalities led to OA diagnosis in most cases; either as the primary finding (30%), or combined with abnormal physical examination or radiographic findings (64%). Owners frequently reported changes in mobility, particularly gait, jumping, and use of stairs. Oral or injectable disease-modifying osteoarthritis drugs were the most common treatments (71%). Feline OA diagnosis and therapeutic monitoring appear to rely heavily on owner-perceived signs; physical examination abnormalities may not be detected. Questioning of owners revealed various observable signs potentially useful in OA detection and monitoring.

2.1.2 Résumé

Signes d'arthrose que perçoivent les propriétaires de chats. Des vétérinaires furent contactés pour identifier des cas d'arthrose féline, et ils ont fourni les informations concernant

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le signalement, la méthode de diagnostic et les traitements administrés à ces chats. Les propriétaires de 50 chats arthrosiques furent sondés pour caractériser les signes d'arthrose liés à la mobilité et l'activité, les soins du corps, le comportement exploratoire, et les habitudes particulières du chat au moment du diagnostic et suite au traitement. L'âge moyen était de 12,0 ans, et plusieurs chats avaient des maladies concomitantes (44 %). Le diagnostic est fondé sur les observations des propriétaires rapportées au vétérinaire compatibles avec de l'arthrose (30 %), ou sur leur recoupement avec les découvertes de l'examen physique ou radiographique (64 %). Les changements au niveau de la mobilité (surtout la démarche, le saut, et la façon de prendre les escaliers) étaient fréquents. Les traitements les plus fréquents étaient les agents structuro-modulateurs (71 %). Actuellement, les observations de changements subtils à la maison de la part du propriétaire sont utilisées pour le diagnostic et le suivi de l'arthrose féline, car des anomalies ne sont pas toujours évidentes lors de l'examen physique. Le questionnement précis des propriétaires a révélé d'autres signes potentiels d'arthrose féline.

2.1.3 Introduction

Osteoarthritis causes chronic pain and disability across mammalian species, but the severity of radiographic signs does not correlate well with expressed pain or functional impairment (Dieppe 2005), thereby hindering diagnosis and therapy. Cats are notoriously difficult subjects when it comes to pain assessment; consequently, feline pain recognition and intervention have historically been deficient (Lascelles and Waterman 1997, Muir, Wiese et al. 2004). Feline OA is particularly challenging for owners and veterinarians to identify, purportedly because signs such as overt lameness are rare (Hardie, Roe et al. 2002, Lascelles 2010). Unalleviated chronic pain is a welfare concern for cats, and functional limitations and pain may contribute to behavior problems (*e.g.*, house-soiling, altered social interactions) (Bennett and Morton 2009). The latter may cause nuisance, property damage, injury (*e.g.*, due to aggression), and loss of the human-animal bond with consequent euthanasia or surrender (Patronek, Glickman et al. 1996).

The radiographic prevalence of feline degenerative joint changes, including OA, is high and increases rapidly with age (Bennett and Morton 2009, Clarke, Mellor et al. 2005, Clarke and Bennett 2006, Godfrey 2003, Godfrey 2005, Gunew, Menrath et al. 2008, Hardie 1997, Hardie, Roe et al. 2002, Lascelles, Hansen et al. 2007, Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011, Zamprogno, Hansen et al. 2010). The infrequent diagnosis of OA-related pain and impaired mobility has raised the question of whether cats lack significant OA pain despite having radiographic signs, or whether they have a species peculiarity making OA pain detection especially difficult (*e.g.*, a lack of lameness as a prominent sign). Reported findings associated with the disease include thickened joints, crepitus, reduced range of motion, objection to manipulation or palpation of affected joints, abnormal gait, anorexia, weight loss, inappropriate elimination, seclusion, and grumpiness toward or avoidance of other household members (human or animal) (Bennett and Morton 2009, Clarke and Bennett 2006, Godfrey 2005, Gunew, Menrath et al. 2008, Hardie 1997, Lascelles, Hansen et al. 2007, Slingerland, Hazewinkel et al. 2011, Zamprogno, Hansen et al. 2010). Overweight cats have an increased risk of non injury-related lameness, which may include OA (Bennett and Morton 2009, Scarlett and Donoghue 1998). Recent studies show differences in demeanor, activity, mobility, self-grooming, and elimination habits between cats with and without OA, and in cats with OA before and after treatment, supporting both the presence of OA pain and disability affecting quality of life, and that signs are apparent to cat owners (Bennett and Morton 2009, Clarke and Bennett 2006, Gunew, Menrath et al. 2008, Lascelles, Hansen et al. 2007, Lascelles, DePuy et al. 2010, Slingerland, Hazewinkel et al. 2011, Zamprogno, Hansen et al. 2010).

History-taking allows the veterinarian to place an emphasis on evaluation of body systems with a suspicion of disease. The gold standard for OA diagnosis is arguably a combination of radiographic and physical examination findings compatible with OA, but even pain detection on a thorough orthopedic examination does not necessarily relate to radiographic OA (67% of apparently painful joints had no radiographic signs of OA in 1 study, and only 36% of joints with radiographic OA were painful in another) (Clarke and Bennett 2006, Lascelles, Hansen et al. 2007). These considerations, combined with the variable nature of feline cooperation with orthopedic examination, highlight the importance of

the anamnesis in veterinary detection of this disease. However, a better understanding of the feline OA signs observed by owners in the home is needed. The goal of this study was twofold: to use a population of cats diagnosed with OA to determine how veterinarians achieved their diagnosis, in particular with respect to the use of owner-reported signs, and to examine owner perceptions of signs that could reflect OA pain. With respect to the latter, we hypothesized that affected cats would demonstrate detectable changes in the categories of mobility and activity, and self-maintenance, social, play and exploratory behaviors, and that these changes could be observed by owners in the home and would contribute to disease detection. Specific objectives were: i) to determine the contribution of owners' reports of signs perceived in the home, relative to the contribution of the physical and radiographic examinations, to OA diagnosis in veterinary practice; ii) to identify additional signs that might be of use in OA detection and monitoring, based on owners' observation of specific behavior changes or other signs noted in the home around the time of OA diagnosis; and iii) to determine what treatments were commonly prescribed by the veterinarian and whether or not owners perceived an effect on these signs.

2.1.4 Materials and methods

Owners of pet cats with a veterinary clinical diagnosis of OA were selected to participate in a phone interview. Information collected included: signalment, method of diagnosis, concurrent medical conditions, treatments for OA, their observations of specific changes in their pet's behavior at OA diagnosis and after treatment. All procedures were approved by the Institutional Animal Care and Use Committee (Rech-1482).

Owners were recruited *via* i) a search of the medical archives of the Université de Montréal's Veterinary Teaching Hospital (VTH), and ii) solicitation of veterinarians at feline-only practices, and at high-volume companion animal practices, in the greater Montreal and Quebec city regions. A letter describing the study purpose and requesting participation was sent to veterinarians, and followed 7 to 10 d later by an initial phone contact, which was followed by further phone, e-mail, and/or in-person contacts as necessary. Veterinarians were asked to provide: contact information for owners of cats they had diagnosed with OA, patient

signalment, and a brief explanation of how the diagnosis was achieved (*i.e.*, whether physical examination abnormalities were identified, whether the diagnosis was confirmed radiographically, or by response to treatment, as well as any other treatment(s) prescribed).

All owner interviews were conducted between March and June 2010. A single interviewer (MK) telephoned each owner, briefly described the purpose of the study, and obtained consent for participation. Interviews were conducted in French or English, according to the respondent's preference. However, the same sequence was followed in both languages, and a standardized script was used to minimize variation that could affect responses. The interview was pilot-tested on cat owners to determine length and comprehensibility of questions; it took approximately 20 min to complete and the only problem identified was that French- and English-speaking respondents interpreted the item for 1 specific behavior, "climbing," differently; it was therefore removed from the survey. Questions on signalment, manner of diagnosis, and treatment were included in order to confirm the information obtained from the veterinarian. All responses were kept confidential, and identifying information was removed prior to data assessment. Interview questions are shown in Table 2.1.1. Descriptive statistics were used to summarize the general characteristics of the population.

2.1.5 Results

Sixteen of 25 veterinary clinics contacted (64%) contributed subjects (n = 53) to the study. Together with patients of the VTH (n = 4), this yielded a total of 56 owners of 57 cats. Of these, 51 owners (91%) were contacted successfully and agreed to participate. Two owners indicated that their cats were recently deceased; their interviews were excluded. One owner completed surveys for 2 cats, yielding a total of 50 surveys.

In the majority of cases (n = 42), the full medical record was not available for review. Veterinarians provided an exact date of diagnosis in 22 cases (44%); owner-reported duration since diagnosis was noted in the remaining cases. In 16 cases, the time from diagnosis to interview was 6 months; in 12 cases, it was 6 months to 1 year; in 9 cases, it was 1 to 2 years; in 13 cases, 2 years. Signalment and concurrent disease information are presented in Table 2.1.2.

Table 2.1.1: Questions and response options for the cat owner interviews.

Question	Response Options
Age at diagnosis	Open-ended
Sex	MI/FI/MN/FS
Breed	Open-ended
Still living in the home	Y/N
Manner of OA diagnosis	Owner history/Veterinary exam/Both
OA treatments and effect	Open-ended
Signs noted by owners at time of OA diagnosis	Open-ended
Presence of specific changes (see list below) at time of OA diagnosis. If yes, describe.	Y/N Open-ended
Responsiveness to treatment of noted changes	Resolved/Improved/No change

Categories of changes

Activity	Sound of footsteps	Appetite
Daily Schedule	Posture	Weight
Jumping	Areas of home used	Vocalization
Stairs	Resting spots	Rubbing behavior
Speed	Time spent resting	Mood
Play/hunting	Use of litter box	Interactions with family members
Agility	Self-grooming	Interactions with family pets
Limping/stiffness	Coat/claw condition	New behaviors
Gait	Scratching/claw sharpening	

Legend: MI — intact male, FI — intact female, MN — neutered male, FS — spayed female.

Table 2.1.2: Age, gender, breed, and presence of concurrent abnormalities for the cats.

Age	12.0 (3.6) years	
Sex	Female spayed	26 (52%)
	Male neutered	24 (48%)
Breed	Domestic	42 (84%)
	Himalayan	2 (4%)
	Persian	2 (4%)
	Cornish Rex	1 (2%)
	Maine Coon	1 (2%)
	Siamese	1 (2%)
	Tonkinese	1 (2%)
Concurrent disease	Overall prevalence	22 (44%)
	Renal disease	11 (22%)
	Diabetes mellitus	6 (12%)
	Cardiac murmur/confirmed disease	7 (14%)
	Hyperthyroidism	5 (10%)
	Ocular disease/visual deficits	3 (6%)
	Possible neurologic disease[a]	1 (2%)
	Allergic dermatitis	2 (4%)
	Feline lower urinary tract disease	1 (2%)
	Hearing loss	1 (2%)
	Diarrhea	1 (2%)
Obesity		5 (10%)

Legend: Age is shown as mean (standard deviation). All other results are shown as number (percent). [a] The neurologic diagnosis that was considered but not confirmed for this cat was intervertebral disc disease.

Aspects of the veterinary clinical evaluation contributing to OA diagnosis are presented in Table 2.1.3. Initial diagnosis was based on a combination of changes in the home reported by the owner (*i.e.*, the anamnesis), and findings on veterinary physical (*e.g.*, pain or palpable joint abnormalities) or radiographic examination for 32 cats (64%). Twelve of these cats (38%) had both radiographic and physical examination abnormalities, 9 (28%) had radiographic signs of OA without physical examination abnormalities (2 were also confirmed by response to treatment), and 11 (34%) had physical examination abnormalities but were not evaluated radiographically (3 were also confirmed by response to treatment). For 15 cats (30%), initial diagnosis was based on owner-reported changes in the absence of physical examination abnormalities, and radiographic evaluation was not performed; in 5 of these (33%), response to treatment was used to confirm the diagnosis. Of 4 cases treated with an oral glucosamine supplement, 1 demonstrated resolution of primary signs (hiding and difficulty climbing stairs), and 3 owners reported general subjective improvements; the remaining cat was treated with meloxicam and the primary complaint resolved (urination and defecation outside the litter box, on beds and sofas). For 3 cats (6%), initial diagnosis was based solely on the veterinarian's examination, in the absence of historical abnormalities observed by the owner; 1 of these cases (33%) was confirmed by both radiographic evaluation and therapeutic response, and 1 by therapeutic response alone. Treatment was administered for OA in 49 cats (98%), and usually was with a single agent (n = 29, 59% of treated cases), but in some cases (n = 21, 43%) was with more than 1 agent (either concurrently or sequentially); specific treatment use is indicated in Table 2.1.4.

Table 2.1.3: Reported use and apparent contributions to osteoarthritis (OA) diagnosis of the various aspects of the veterinary clinical evaluation.

Aspect of evaluation	Cases assessed	Abnormal finding(s)		Cases affected
Physical examination[a]	50 (100%)	All		26 (52%)
		Observable	Limping/stiffness	5 (10%)
			Postural abnormality	3 (6%)
		Palpable	Pain upon palpation	9 (18%)
			Palpable changes other than pain	10 (20%)
			Joint swelling	6 (12%)
			Joint laxity	4 (8%)
			Crepitus	3 (6%)
			Muscle atrophy	2 (4%)
			Decreased range of motion	1 (2%)
		Unspecified		6 (12%)
Owner report	50 (100%)	Suspicious for OA	History	47 (94%)
		Supporting diagnosis	Treatment response	12 (24%)
Radiographs	22 (44%)	Change(s) compatible with OA		22 (44%)

Legend: Results presented as number (percent of cases diagnosed). [a] Details of how the physical examination was conducted were not collected from veterinarians. It is therefore not known whether all aspects of the orthopedic examination (*e.g.*, gait/posture evaluation, joint palpation and manipulation) were performed in all cases.

Table 2.1.4: OA treatments administered.

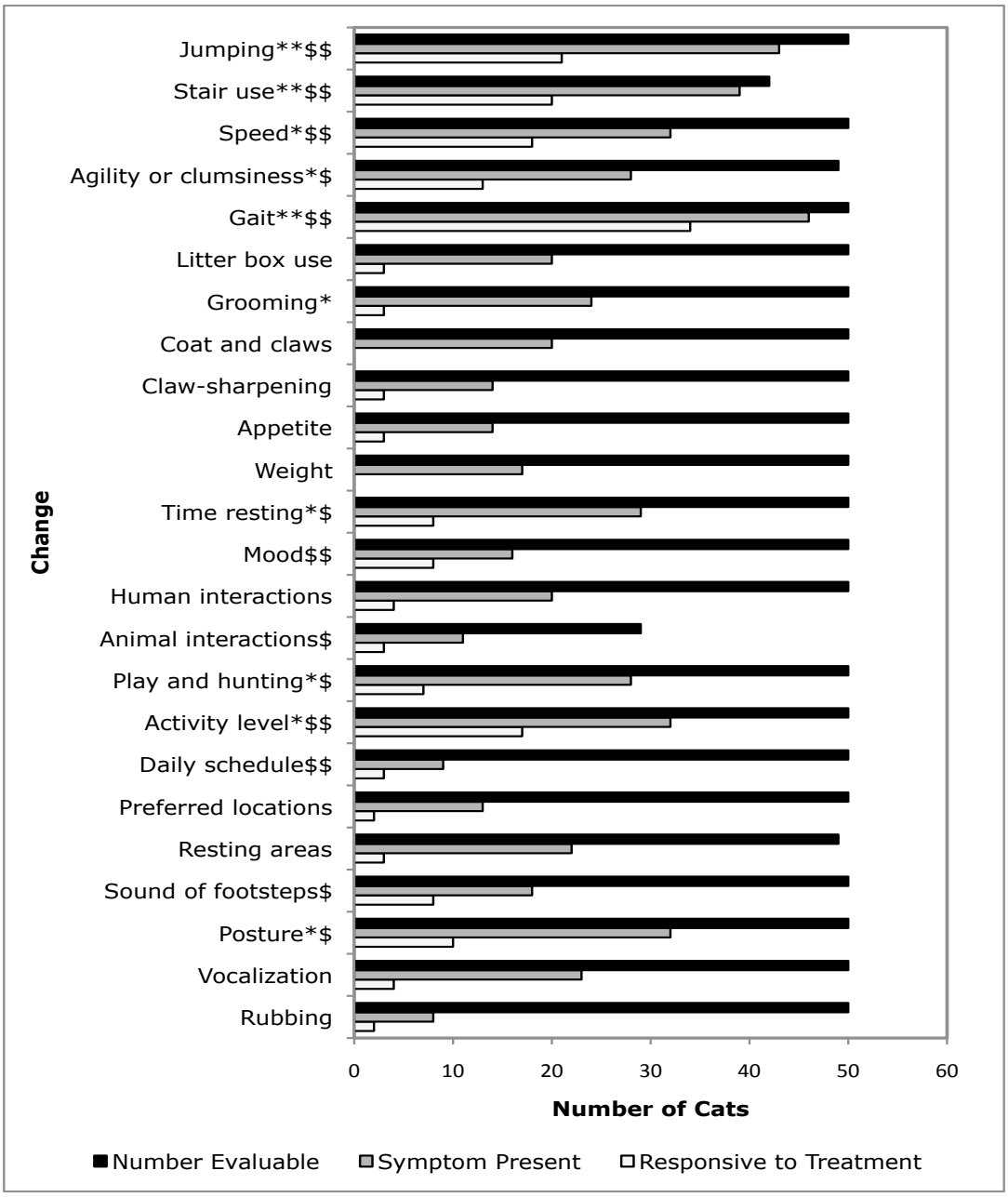
Treatment	Details	Number (%)	Monotherapy (%)
DMOADs[a]		35 (71%)	17 (59%)
	Injectable PSGAGs[b]		
	Injectable pentosan PS[c]		
	Oral glucosamine supplement		
NSAIDs[d]		16 (33%)	8 (28%)
	Meloxicam		
	Tolfenamic acid		
Dietary therapy	MediCal Mobility Support®	12 (24%)	4 (14%)
Other	Gabapentin	3 (6%)	0
	Glucocorticoids	3 (6%)	0
	Laser	1 (2%)	0
	Herbal supplement	1 (2%)	0

Legend: For specific treatments, percentages shown are of total number treated (Number) or of total number treated with a single agent (Monotherapy).

[a] DMOADs: Disease-modifying osteoarthritis drugs; [b] PSGAGs: Polysulfated glycosaminoglycans (Adequan®; Novartis Animal Health, Mississauga, Ontario); [c] Pentosan PS: Pentosan polysulfate (Cartrophen Vet; Arthropharm Services, Embrun, Ontario); [d] NSAID: Non-steroidal anti-inflammatory drug.

Figure 2.1.1 summarizes owner perceptions of prevalence of signs and treatment responsiveness. **Gait** changes included limping or stiffness (which was more severe after rest in 8 cases), and changes in limb carriage or appearance (n = 15). Owners also reported reductions or cessation in **jumping** (n = 22) and **stair use** (n = 8) as well as changes in the ways in which cats performed these activities (n = 27, *n* = 32, for jumping and stair use respectively), such as hesitation, stumbling or falling, or doing several small jumps instead of 1 large one, or a few stairs at a time instead of the entire flight. Some cats (n = 6) were also reported to have begun "asking" for help (by vocalizing, staring, or tapping with a paw).

Figure 2.1.1: Owner-perceived changes associated with OA: sign prevalence and perception of response to therapy.



Legend: ** change present in ≥ 75% of OA-affected cats; * change present in ≥ 50% of OA-affected cats; $$ perceived sign responsiveness to treatment in ≥ 50% of treated cats; $ perceived sign responsiveness to treatment in ≥ 25% of treated cats.

Changes in **litter box use** included elimination outside the box (n = 11), which was sometimes interpreted as being due to an inability to reach the litter box prior to elimination (due to urgency) or reluctance to negotiate stairs en route, as well as apparent difficulty maneuvering in the box (n = 9). General decreases in **grooming** were reported (n = 15), but also decreases (n = 7) and an increase (n = 1) in grooming of particular areas, and 1 cat started leaning against something while grooming. Owners noted various **coat** changes (n = 14), and **claws** that were longer, brittle, or more dull (n = 5), in addition to decreases in (n = 8) or changes in the manner of (n = 5) **claw-sharpening** (*e.g.*, on horizontal instead of vertical surfaces). **Appetite** changes included reductions (n = 8; it increased in 2 of these after treatment), increases (n = 2), and increased variability (n = 2). Where **time resting** was affected, it was generally (n = 27) increased. Social behavior changes included a poorer **mood** (n = 9), occasional increased (n = 1) or decreased (n = 1) general fearfulness, increased (n = 2), or decreased (n = 1) friendliness to strangers, and increased (n = 9) or decreased (n = 7) **interactions** with family members (*e.g.*, following them more, sleeping with them more or less). Several cats (n = 14) reacted to being picked up or to being touched in certain areas (*e.g.*, near joints diagnosed with OA). Changes in **interactions with household animals** were reported to be decreased playfulness and tolerance, and increased frequency of being chased or picked on, rather than chasing or picking on the other animals. Changes to **play and hunting** (n = 24) were usually reductions or cessation; others were playing in a recumbent position (n = 2), or no longer following birds in the windows (n = 1). Changes in overall activity and habit changes were usually decreased **activity level** (n = 26), less time spent outside (n = 6), and seeking heat/sun (n = 4). Miscellaneous changes included altered overall character or frequency of *vocalizations,* increased vocalizing at the owner (n = 6), meowing from other parts of the house or when moving about (n = 6), or decreased meowing to be let out (n = 1). Changes of *posture* were described as a change in preferred positions (n = 7; *e.g.,* sitting or lying more), or asymmetry or other abnormalities of posture (n = 18; *e.g.,* hunched appearance, legs held loosely rather than tucked against the body, holding a paw up). **Head- or body-rubbing** behavior changes were general reductions (n = 3), an increase (n = 1), a change in targets (n = 1), or clumsiness (n = 1). Other changes volunteered by owners (n = 6) were: less adventurous, lying down/getting up slowly or with difficulty, hiding, and worsening of signs in damp weather.

2.1.6 Discussion

Our findings suggest that owners perceive particular signs linked to feline OA, in the home setting, and owner-perceived signs contribute substantially to the diagnosis of feline OA in veterinary practice in Quebec. This is consistent with other reports that have found owners to be capable of identifying signs of feline OA in the home and response to therapy (Bennett and Morton 2009, Clarke and Bennett 2006, Gunew, Menrath et al. 2008, Lascelles, Hansen et al. 2007, Zamprogno, Hansen et al. 2010).

Our relatively high response rates from veterinarians and owners, and the gender and breed distribution of the cats in our sample are consistent with reports in the literature and suggest a good representation of pet cats visiting veterinarians in our area (Chu, Anderson et al. 2009, Toribio, Norris et al. 2009). A high mean age is consistent with selection based on the presence of OA, which affects geriatric animals preferentially (Clarke, Mellor et al. 2005, Clarke and Bennett 2006, Godfrey 2005, Hardie, Roe et al. 2002, Slingerland, Hazewinkel et al. 2011), and is compatible with the high prevalence of concurrent geriatric diseases. We found inconsistencies in appetite and weight changes, compatible with previous reports (Bennett and Morton 2009, Scarlett and Donoghue 1998), and unsurprising in view of veterinarian recommendations for diet changes and the presence of concurrent geriatric diseases affecting weight and appetite.

Small numbers of cats were diagnosed with OA at each participating clinic, despite recent research reporting a high prevalence of feline degenerative joint disease including OA (Bennett and Morton 2009, Clarke, Mellor et al. 2005, Clarke and Bennett 2006, Godfrey 2003, Godfrey 2005, Gunew, Menrath et al. 2008, Hardie 1997, Hardie, Roe et al. 2002, Lascelles, Hansen et al. 2007, Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011, Zamprogno, Hansen et al. 2010). This may have been due to a lower prevalence in our population; alternatively, it could indicate that feline OA continues to be challenging to diagnose in private veterinary practice. The diagnosis of most cases in this study was based at least in part on owner-reported abnormalities suggestive of OA (n = 47, 94%); 33 of these cases (70%), were confirmed by radiographic and/or physical examination findings, or a clear

response to treatment. It was rare for veterinarians to detect OA based on their physical examination alone, in the absence of reported historical abnormalities; conversely, they were unable to identify physical examination abnormalities in several cats with both historical and radiographic findings supportive of OA. Although it is possible that radiographic findings were not the cause of the reported signs in some of these cases, it seems prudent to conclude that a lack of musculoskeletal abnormalities upon physical examination does not preclude the presence of clinical OA. Hence, the variable correlation of palpation with radiographic OA findings previously reported (Clarke and Bennett 2006, Lascelles, Hansen et al. 2007) may not simply reflect a weak relationship between structural joint changes and clinical (*i.e.*, affecting pain and function) OA; physical examination abnormalities (*e.g.*, pain) may also be particularly difficult to confirm in the cat.

The most common owner-reported changes in this study related to mobility (gait, jumping, and stair use), followed by changes in activity level, time spent resting, and self-grooming, and changes in social behavior such as interactions with humans and animals and mood, and litter box use, consistent with previous studies (Bennett and Morton 2009, Clarke and Bennett 2006, Gunew, Menrath et al. 2008, Lascelles, Hansen et al. 2007, Lascelles, DePuy et al. 2010, Slingerland, Hazewinkel et al. 2011). Additionally, our findings support other proposed signs of OA such as vocalization and objections to handling, and changes in resting areas, play and hunting behavior, and posture (Hardie 1997). In general, the usefulness of subjective behavior parameters in addition to more objective measures, primarily of mobility such as gait, jumping, and use of stairs, is supported.

Interestingly, gait changes were the most common sign reported by owners, who also perceived them to be responsive to therapy. Our study design may have inadvertently selected for particularly attentive owners or limping cats (perhaps because this sign is more obviously suggestive of OA than are many others). Gait changes could be common at home but rarely observed during examination; however, one might then expect owners to report them more frequently. Veterinarians in our study were indeed far more likely to detect abnormalities on palpation than to observe stiffness or limping during their examination, despite many of the owners of examined cats having noted gait changes at home. Notwithstanding these

possibilities, our findings strongly suggest that gait changes may be useful in the diagnosis of at least a subpopulation of OA-affected cats.

Some of the OA diagnoses based on owner reports and unconfirmed by abnormal radiographic or physical examination findings (or a clear response to treatment reported to the veterinarian) could have been incorrect; veterinarians were not asked to indicate their degree of certainty in these cases. The retrospective nature of the study may also have introduced recollection bias; questioning about specific behaviors was expected to reduce subjectivity. Despite these potential limitations, our findings correspond well with previous reports, including a recent study comparing groups of OA-affected and unaffected cats for possible OA signs in the home (Zamprogno, Hansen et al. 2010), and a cross-sectional study in 100 cats with OA (Slingerland, Hazewinkel et al. 2011). This supports their validity and makes additional signs uncovered in our study worthy of further investigation.

There are relatively few products licensed for the chronic treatment of feline OA. Unlike non-steroidal anti-inflammatory drugs (NSAIDs), the analgesic effects of glucosamine, pentosan polysulfate (PS), and polysulfated glycosaminoglycan (PSGAG) alone or in combination with other nutraceutical(s), have not been clearly established (Lascelles, DePuy et al. 2010, Wandel, Jüni et al. 2010), and their effects on OA signs may be *via* other mechanisms. In our sample, frequent use of glucosamine and PSGAG may have reflected fear of adverse effects of long-term NSAID use in geriatric cats or in those with concurrent diseases (Lascelles 2010). The infrequent use of therapeutic diets was likely due to their having become available only very recently. Although information was collected on owners' perception of treatment effects on the OA signs they observed, the nature of the treatments used, the potential for recall bias, and the lack of veterinary confirmation in some cases make us unable to draw conclusions regarding treatment effects.

Although behavior changes in the home appear pertinent in the diagnosis of feline OA, the sensitivity and specificity (*i.e.*, predictive validity) of these signs for use in the detection and monitoring of this disease have not been determined. Our study was performed to collect information on the methods of feline OA diagnosis in private practice, and on owner-perceived signs in the home that may be associated with OA. It did not include a control

population of owners of age-matched, unaffected cats, and it included cats with concurrent diseases (*e.g.*, cardiac disease, renal disease, hyperthyroidism, and ocular disease could cause weakness, diabetic neuropathy-associated pain, and visual deficits, affecting apparent mobility, activity level and social interactions) (Kittleson 2005, Mooney 2005, Nelson 2005, Polzin, Asborne et al. 2005). Future research is therefore needed to determine whether the identified OA signs bear any confounding relationship to other age-related problems, such as geriatric diseases other than OA, cognitive decline, and sensory deficits. Prospective, placebo-controlled, blinded studies will also be needed to confirm the therapeutic responsiveness associated with these OA signs. This will help to improve the certainty of OA diagnosis by practitioners, when physical examination findings are negative or equivocal.

Feline OA diagnosis remains challenging for practitioners; it continues to be relatively infrequent, despite a growing body of research supporting the disease's prevalence and importance in aged cats. Pending future studies confirming the specificity of these signs, owners of cats at risk for OA should be questioned carefully about subtle behavior changes in the home, particularly those relating to mobility (*e.g.*, gait, stair use, and jumping changes) at the veterinary appointment. This is especially important because some owners may not volunteer this information without prompting, thinking it is due to "normal aging," and because a thorough orthopedic examination may not adequately detect OA pain in many cats. Careful examination of gait and posture, as well as palpation and manipulation of the joints should also be performed in at-risk cats, particularly since some owners of cats with OA may not observe abnormalities in the home. Where physical examination abnormalities are found, or where they are lacking but owners report possible OA signs, further diagnostics and/or a therapeutic trial should be considered, in order to improve detection, diagnostic certainty, and treatment of feline OA.

2.1.7 Acknowledgments

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2.2 Development and Preliminary Validity and Reliability of the Montreal Instrument for Cat Arthritis Testing, for Use by Caretaker/Owner, MI-CAT(C), *via* a Randomized Clinical Trial

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2.2.1 Abstract

Challenges in the clinical assessment of feline osteoarthritis-related pain and disability impede diagnosis and treatment of the disease. A pain scale was developed for use by cat owners and caretakers, the Montreal Instrument for Cat Arthritis Testing - Caretaker/Owner (MI-CAT(C)). Following content validation and a pilot assessment (n = 11 cats with and without OA) of MI-CAT(C)-v1 reliability and validity, a randomised, double-blinded, placebo-controlled, crossover clinical trial was conducted; meloxicam efficacy in 54 OA-affected cats was evaluated using the MI-CAT(C)-v2 and locomotor activity monitoring (AM). The intra-class correlation coefficient was 0.81 for total scale intra-rater reliability, and 0.64 for inter-rater reliability; secondary owners tended to have more trouble completing the scale than did primary owners. Internal consistency assessed by Cronbach's alpha was > 0.70 for the total scale, but < 0.70 for subscales and subcategories. Compared to reference level, MI-CAT(C)-v2 score decreased by 17.56% with meloxicam ($P < 0.05$) and increased with age ($P < 0.01$). Night-time AM (NAM) was lower than daytime AM ($P < 0.0001$). Actimetry increased by 23.83% with meloxicam treatment ($P < 0.0001$). MI-CAT(C)-v2 scores correlated negatively with log NAM ($Rho_P = -0.36$, $P = 0.0074$) and positively with age (Rho_P

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= 0.43, P = 0.0011). These preliminary findings support the reliability and validity of the MI-CAT(C)-v2 when completed by the primary owners of OA cats. However, questions remain regarding item comprehension and internal scale structure/internal consistency. Further refinement and testing should include a comprehension analysis and exploratory factor analysis in a larger sample of cats, as well as evaluation of sensitivity/specificity to OA status in a sample of cats with and without OA, testing of responsiveness to other OA therapies and ability to distinguish treatment from placebo, and finally, development of guidelines for clinical use, such as determination of the minimum clinically important difference in scale score and thresholds for determining OA *vs.* non-OA status.

2.2.2 Introduction

Feline osteoarthritis (OA) is an important cause of pain and physical disability (Bennett, Zainal Ariffin et al. 2012, Lascelles and Robertson 2010). Although radiographic prevalence is high, particularly in aged animals (Lascelles, Henry III et al. 2010), clinical OA diagnosis is relatively infrequent, and remains challenging (Lascelles and Robertson 2010). Possible reasons include an insidious nature of the disease, subtle signs easily confounded with other signs of aging, and low prevalence and/or poor concordance of physical examination findings such as lameness, palpable abnormalities or pain, with radiographic signs of OA (Bennett, Zainal Ariffin et al. 2012, Lascelles, Dong et al. 2012). Veterinary diagnosis relies heavily on owner-reported abnormalities (Klinck, Frank et al. 2012, Lascelles 2010). There is evidence that cats with OA show improvement when treated with the non-steroidal anti-inflammatory drugs (NSAIDs), meloxicam (Bennett, Zainal Ariffin et al. 2012) and robenacoxib (Giraudel, Gruet et al. 2010), as well as with feline anti-nerve growth factor antibody (Gruen, Thomson et al. 2016), or a therapeutic diet (Lascelles, DePuy et al. 2010). Treatment has been reported to improve mobility (*e.g.* jumping) and activity (Guillot, Moreau et al. 2013, Lascelles 2010), lameness/stiffness (Gunew, Menrath et al. 2008, Lascelles, Hansen et al. 2007), mood (Bennett and Morton 2009, Giraudel, Gruet et al. 2010, Gunew, Menrath et al. 2008), and self-grooming (Bennett and Morton 2009).

Individual differences in pain experience and expression, and variable observer interpretive capabilities, complicate assessment. Reported objective measures include: peak vertical ground reaction force (PVF) (Schnabl and Bockstahler 2015), von Frey punctate tactile withdrawal threshold (VF) (Guillot, Moreau et al. 2013), telemetric locomotor activity monitoring (AM) (Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2007), thermographic imaging (Vainionpää, Raekallio et al. 2013), video fluoroscopic kinematics (Guillot, Gravel et al. 2015), functional bio-imaging (Guillot, Chartrand et al. 2015) and response to mechanical temporal summation (Guillot, Taylor et al. 2014). However, such objective measures may not be clinically feasible. Pain scales are standardised subjective measures facilitating comparison within and between individuals (Robertson 2008). One such scale is a client-specific outcome measures questionnaire (CSOM), which identifies activities affected by the disease and rates the degree to which they are affected, permitting within-individual comparisons over time (Lascelles, Hansen et al. 2007). Two standardised multi-item numerical rating scales (NRSs) have also been reported. One was able to distinguish cats with and without OA (Benito, Depuy et al. 2013), and both were reported to detect treatment effects (Bennett and Morton 2009, Gruen, Griffith et al. 2015). Only the CSOM (Lascelles, Hansen et al. 2007) and the feline musculoskeletal pain index (FMPI) (Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Gruen, Thomson et al. 2016) have been tested in placebo-controlled, blinded studies. Challenges remain in distinguishing different severities of OA (Benito, Hansen et al. 2013), and treatment from placebo effect (Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015). Pain scales require validation to confirm effective and consistent measurement of the condition of interest (*e.g.* feline OA), in the target context (*e.g.* assessed by the owner, in the home) (Streiner and Norman 2008). This comprises "content" (expert assessment of completeness and representativeness), "face" (content and format acceptability) (Crellin, Sullivan et al. 2007, Streiner and Norman 2008), "criterion" ("concurrent" or "predictive" comparison with a "gold standard") (Streiner and Norman 2008) and "construct" validation, which is used when no gold standard exists (*e.g.*, in OA pain) for direct quantification (between-groups comparisons, response to treatment, and convergence with and/or divergence from related and distinct constructs, respectively (Crellin, Sullivan et al. 2007, Streiner and Norman 2008)). "Reliability" (degree of freedom from measurement error) must also be assessed, including inter- (*i.e.* consistency between evaluators) and intra-rater

(*i.e.* repeatability within an evaluator) reliability, and internal consistency (interrelatedness of scale items) (Crellin, Sullivan et al. 2007, Streiner and Norman 2008).

We hypothesised that OA pain status and response to treatment with a NSAID, meloxicam, could be reliably detected using a pain scale based on the owner's perception of cat behaviours/abilities relating to agility/mobility, physical condition, and social, play, exploratory and self-maintenance behaviours. This study consisted of the development and preliminary validation of such a scale. Specific objectives included comparisons of scale ratings: 1) before and after NSAID treatment, in comparison to a placebo, and 2) with the results of an objective measure; 3) repeated by the same owner at baseline and 4) between different owners at one time point. An additional objective was 5) to question owners on the clarity of scale items.

2.2.3 Materials and methods

2.2.3.1 Ethical approval

The University of Montreal Institutional Animal Care and Use Committee (IACUC) approved scale development and preliminary reliability/content validity testing protocols (#Rech-1482). North-Carolina State University's IACUC approved the clinical trial study protocol (assessment of reliability/construct validity) (#11-102-O).

2.2.3.2 Scale development and preliminary validation

Preliminary content for the Montreal Instrument for Cat Arthritis Testing – Caretaker/Owner (MI-CAT(C)) was developed based on a review of the literature (Bennett and Morton 2009, Clarke and Bennett 2006, Godfrey 2005, Gunew, Menrath et al. 2008, Hardie, Roe et al. 2002, Lascelles 2010, Lascelles, Hansen et al. 2007, Lascelles and Robertson 2010, Lascelles, DePuy et al. 2010, Scarlett and Donoghue 1998, Zamprogno, Hansen et al. 2010) and the authors' collective experience. Fifty-two scale items were generated consisting of statements falling into the following subcategories: Agility/mobility, Physical condition, Social, play, and exploratory behaviours, and Self-maintenance

behaviours. Items were divided into two subscales expected to be consistent with: 1) absence of OA (Subscale 1; n = 26), and 2) presence of OA (Subscale 2: n = 26).

Internal (n = 3; including MPK and ET; one pain and two behaviour specialists at the Faculté de médecine vétérinaire of the Université de Montréal) and external (n = 4; international experts in feline pain, including BDXL) reviewers validated scale content. Each rated scale items on their clarity and importance (possible ratings: one = poor, two = fair, three = good), and commented on specific items, and on general scale construction and content. Scale content was also compared with abnormalities reported in a survey of the owners of cats diagnosed with OA (Klinck, Frank et al. 2012). Modifications to scale format and response options, and to individual items (additions, deletion, wording changes), based on the results, produced the MI-CAT(C)-v1 (see **Appendix** A), with 27 items in Subscale 1 and 32 items in Subscale 2.

The MI-CAT(C)-v1 was preliminarily evaluated *via* a pilot, laboratory study of seven cats with naturally-occurring OA, and four non-OA cats; cats were group-housed in a room with access to toys, perches, hiding places, and a large window. Two animal care attendants familiar with the cats (reference observer = weekday caregiver; secondary observer = weekend caregiver) performed concurrent scale assessments twice, on Days 0 and 7, permitting intra- and inter-rater reliability assessments, an internal consistency evaluation, and comparison of OA and non-OA scale scores. Individual item analysis considered the following criteria to flag items for potential removal from the scale (in order of importance): 1) a tendency for negative correlations with other subscale items, and 2) high numbers of missing or "Don't know/Does not apply" responses, 3) lack of response variability, 4) intra-rater reliability no better than fair, and 5) inter-rater reliability no better than slight (see below for interpretation of reliability coefficients). Where flagged items were considered particularly difficult to evaluate in the laboratory context, they were retained in the scale. The revised scale had 18 items in Subscale 1 (three categories: 1) Agility, 2) Social, play and exploratory behaviours, and 3) Self-maintenance), and 20 in Subscale 2 (three categories: 1) Agility, 2) Self-maintenance and 3) Physical condition). See **Appendix A** for the MI-CAT(C)-v2.

2.2.3.3 Randomised, double-masked, placebo-controlled, crossover clinical trial: scale construct and face validity, and reliability assessment

2.2.3.3.i Animals

Client-owned, adult cats with naturally-occurring chronic musculoskeletal disease were recruited as previously described (Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015), based on owner-reported mobility/activity impairment of at least 3 months' duration, and a history of a veterinary diagnosis or suspicion of OA. Cats had to be over 1 year of age, weigh more than 1 kg, live indoors, be apparently healthy aside from OA, and not be receiving any anti-inflammatory treatment. Screening (Day 0) consisted of general, orthopaedic, and neurologic physical examinations, as well as laboratory screening (complete blood count, serum chemistry and T4, urinalysis with sediment evaluation), and orthogonal radiographs of all axial and appendicular joints. Owner-perceived mobility impairment, detectable pain in at least two joints having radiographic evidence of OA, and absence of systemic illness (except for stable chronic renal disease up to IRIS stage 2) were inclusion requirements. At Day 0, cats were fitted with collar-mounted telemetric activity monitors (Actical Z, Philips Respironics, Bend, Oregon, USA), with counts made at 1-minute intervals and numeric amplitude (0 to infinite, no unit) based on intensity, as previously described (Gruen, Griffith et al. 2015, Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2008).

2.2.3.3.ii Study design

The first 2 weeks of the study (Baseline, Day 0-14) were not blinded; all cats received oral placebo (0.07 mL/kg/d), to acclimate participants to medication administration, wearing the activity monitor, and record keeping. Following Day 14, treatment/placebo assignment and design details were masked from owners/investigators. A crossover design with washout phase was begun on Day 15. Randomised group allocation was performed according to pre-determined randomisation tables, after stratifying cats into high and low impairment groups on the basis of CSOM scores (Gruen, Griffith et al. 2015). From Day 15-35 (Treatment period 1), cats either received oral meloxicam (Metacam 0.5 mg/ml Oral Suspension, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri, USA) at a dose of 0.035 mg/kg/d (Group A)

or volume-matched placebo formulated identically except for the absence of meloxicam (Group B); from Day 36-56, all cats received placebo (Washout), and from Day 57-77 (Treatment period 2), cats received the opposite treatment, placebo (Group A) or meloxicam (Group B). All outcome data for each period were collected before treatments were changed for the subsequent period. Meloxicam is an NSAID approved for chronic use in musculoskeletal disease in cats in Europe, Canada, Australia, and New Zealand, though only for a single injection for surgical pain in the USA.

2.2.3.3.iii Outcome measures

The MI-CAT(C)-v2 was completed by the primary owner on Days 0, 15, 36, 57 and 78, and by a second household member (where possible) on one occasion. Activity monitoring (actimetry) data for each period were collected through Days 14, 35, 56 and 77. Intra-rater and internal consistency reliabilities were assessed based on the Days 0 and 15 evaluations; inter-rater reliability was assessed based on concurrent evaluations by the primary and secondary owners (the evaluation day varied and was selected based on owner convenience, for each pair). Construct validity consisted of assessing response to treatment with meloxicam *vs.* placebo, and convergence of MI-CAT(C)-v2 scores with AM data and age (at Baseline/Day 15). Finally, face validity was assessed by asking owners to indicate whether each item was clear/easy to understand or not, the first time they completed the scale.

2.2.3.3.iv Statistical methods

2.2.3.3.iv.a Expert review and pilot study

Based on the expert review, the medians of the importance and clarity scores (each having a maximum score = 3) were calculated, as well as the medians of the total score (sum of importance and clarity scores; maximum score = 6), for each scale item. For the pilot study, item intra- (Days 0 and 7; both observers) and inter-rater (Day 0; reference and secondary observers) reliability were evaluated using Kappa (or percentage agreement where Kappa could not be calculated) for individual items, and weighted Kappa and Spearman's Rho (Rho_S) for subscale totals. The latter were the counts of "Yes" responses for each subscale. Agreement based on Kappa was interpreted as follows: < 0.00 = poor, 0.00-0.20 = slight, 0.21-

0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial, 0.81-1.00 = almost perfect (Landis and Koch 1977). Where Kappa could not be calculated, percentage agreement < 50% (*i.e.*, agreement less than that expected due to chance for dichotomous outcomes) was considered poor. Spearman's Rho correlations were also used to assess internal consistency (*via* examination of inter-item correlation matrices for each of the two subscales, and *via* correlation of Subscale 1 and 2 total scores. Comparison of Subscale 1 and 2 total scores for OA *vs.* non-OA cats was accomplished using Wilcoxon Rank Sum tests (WRS).

2.2.3.3.iv.b Clinical trial

Scale total score calculation was based on the number of abnormal responses ("No" for Subscale 1, "Yes" for Subscale 2) divided by the total number of "Yes" and "No" responses (see **Appendix A**, MI-CAT(C)-v2); hence, it was based on the answered items only. Pairwise deletion was used to handle missing MI-CAT(C)-v2 data; no imputation method was applied. Subcategory, subscale, and total scale intra-and inter-rater reliabilities were assessed using intra-class correlation coefficients (ICC) based on a single rating, absolute agreement, one-way random effects model; interpretation was as follows: < 0.40 = poor, 0.40-0.59 = fair, 0.60-0.74 = good, 0.75-1.00 = excellent (Cicchetti 1994). Internal consistency was evaluated using Cronbach's alpha, with a result of between 0.70 and 0.90 considered desirable (Streiner and Norman 2008, Tavakol and Dennick 2011), and *via* examination of inter-item correlations and item-subscale total correlations for each subscale. Subscale and subcategory alphas were calculated based on individual items, while scale total Cronbach's alpha was calculated based on subcategory scores (proportion of abnormal responses) because the large number of scale items could be expected to inflate alpha. Total scale scores from the assessments performed at the end of each treatment period (*i.e.* at 36 or 78 days of study) were retained for analysis with a generalised linear mixed-effect model (GLMM). Because the response variable was a percentage, data were assumed to follow a beta distribution function (*i.e.* bounded at 0 and 1), which was linked to the linear predictors through a logit function. The fixed-effect predictors of this model were age (in days, a covariate), treatment, period, and the cat's CSOM score at the time of recruitment. Sequence of treatment (see Figure 2.2.1), and cat nested within sequence were used as random factors. An unstructured covariance matrix parametrised

through its Cholesky root was used to obtain at least a positive semidefinite estimate of the variances and covariances of the fixed effects, a procedure that was achieved through the use of the classical sandwich estimator (SAS Institute Inc 2015). Activity monitoring data were expressed as intensity count summations over both daily 12-h periods of assessment (night-time AM (NAM): 6:00 pm to 5:59 am; daytime AM (DAM): 6:00 am to 5:59 pm). The fixed effects of treatment, treatment period, and the night-day phase (*i.e.*, NAM or DAM) on 12-h actimetry summations were assessed with a GLMM for a log-normally distributed outcome variable, with cat nested within treatment sequence as a random variable, and a first-order, autoregressive moving average covariance matrix to model the cat-specific residual random variation over time. Relationships between MI-CAT(C)-v2 scores, age and log AM (NAM and DAM) were assessed *via* Pearson correlations, single and multiple regression analyses, and a collinearity analysis. All analyses were two-tailed with an α-level of 0.05; a Bonferroni adjustment was used to correct for multiple comparisons in the *post hoc* analysis of AM, but no such correction was made for the other convergent validity analysis. Analyses were performed using statistical software (SAS system, version 9.4, SAS Institute Inc.; IBM SPSS Statistics for Macintosh, version 22, IBM Corp).

2.2.4 Results

2.2.4.1 Expert review and pilot study

In the expert review, all individual item median clarity and importance scores were ≥ 2/3, and all median total scores were ≥ 5/6. A variety of comments were made regarding overall scale format, response options, and individual items (details not presented).

In the pilot study, MI-CAT(C)-v1 individual item inter- and intra-rater reliability were highly variable (inter-rater reliability: Kappa range = -1.00-1.00, percentage agreement range = 36-100%; intra-rater reliability: Kappa range = 0.083-1.00, percentage agreement range = 33-100%). Inter-rater and intra-rater reliabilities for subscale totals are presented in Table 2.2.1. Subscales 1 and 2 were inversely correlated (Rho_S = -0.70, P = 0.016). Neither subscale distinguished OA from non-OA cats (P > 0.20).

Sixteen scale items had high numbers of non-responses (*i.e.*, in ≥ 3/11 cases) for the reference observer; for the second observer, more items were affected in this way (data not presented). Frequent negative correlations with other subscale items were detected in 20 scale items. Twenty-four scale items demonstrated little response variability (*i.e.*, ≤ 1 case per evaluation day differing from the other cases). Table 2.2.2 summarises the results of the item analysis for each subscale.

Table 2.2.1: Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – version 1 Subscale 1 and 2 intra- and inter-rater reliabilities, based on animal caretaker assessments of 11 laboratory cats.

Scale component	Intra-rater reliability		Inter-rater reliability	
assessed	wKappa	Rho_S	wKappa	Rho_S
Subscale 1	0.80	0.98 ($P < 0.0001$)	0.28	0.81 ($P < 0.0001$)
Subscale 2	0.68	0.83 ($P = 0.0016$)	0.35	0.76 ($P < 0.0001$)

Legend: Intra-rater reliability was evaluated based on two assessments, one week apart, by the reference observer (most familiar with the cats); inter-rater reliability was evaluated based on same day assessments by the reference and a second observer. wKappa = weighted Kappa; Rho_S = Spearman's Rho.

Table 2.2.2: Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – version 1 item response characteristics in the pilot study (n = 11) that were considered as potential causes for item rejection or revision, based on two assessments (Days 0 and 7), each by two animal care attendants.

	Criteria prompting item review	Number of items		
		Affected	Rejected	Retained
Subscale 1	Negative correlations with other scale items[a]	10	9	1
	Missing/"Don't know" entries[b]	11	5	6
	Lack of response variability[c]	10	3	7
	Intra-rater reliability = slight to fair[d]	4	3	1
	Inter-rater reliability = slight to none[e]	9	3	6
Subscale 2	Negative correlations with other scale items[a]	10	8	2
	Missing/"Don't know" entries[b]	5	0	5
	Lack of response variability[c]	14	7	7
	Intra-rater reliability = slight to fair[d]	5	2	3
	Inter-rater reliability = slight to none[e]	3	2	1

Legend: [a] Based on the inter-item correlation matrix for the reference observer. [b] In ≥ 3/11 cases (for the reference observer on at least one evaluation day). [c] Defined as ≤ 1 case differing from the others (for the reference observer on at least one evaluation day). [d] Based on: 1) Kappa for at least one evaluator < 0.40, or 2) Kappa non-evaluable for both evaluators. [e] Based on: 1) Kappa < 0.20, or 2) (if Kappa could not be calculated) percentage agreement < 50%.

2.2.4.2 Clinical trial

2.2.4.2.i Animals

Sixty-six client-owned cats were recruited and completed screening. Data for 12 were excluded due to adverse events (vomiting: n = 3; acute renal injury: n = 2; seizure: n = 1; unusual behaviour: n = 1) or noncompliance with the study protocol (n = 5). Six of seven cats with adverse events developed them during meloxicam treatment; one during placebo (unusual behaviour, consisting of running about wildly for a few minutes on one day, and urinating on the floor followed by hiding under the bedcovers the next day; treatment was stopped and the behaviour returned to normal). The remaining 54 cats were 6-21 years old (mean: 12.4 years); 24 were castrated males (44.4%) and 30 were spayed females (55.6%). Of these, 40 (74.1%) had access to stairs. There were 51 owners (three owners each with two cats), 80.4% female and 19.6% male; median age was 41 years (range: 25-70) for the 49 owners for whom age was available.

2.2.4.2.ii Missing data

Missing MI-CAT(C)-v2 data (based on all evaluation days and owners) was attributable to missing entries and to "Don't know/Does not apply" responses. Missing entries amounted to ≤ 1.32% for any individual scale item, and 0.33% for all items combined. Owner selection of "Don't know/Does not apply" responses were more common, with an item median of 2.14% (range: 0-23.36%), amounting to 4.48% "Don't know/Does not apply" responses for the entire data set. The four MI-CAT(C)-v2 items relating to stair use had relatively high numbers of "Don't know/Does not apply" responses (21.71-23.36%), and two other items had 10.86% ("My cat can easily scratch their head or neck with either hind foot") and 7.57% ("My cat climbs vertical surfaces (such as a cat tower/furniture/trees)"); all other items had ≤ 5.26% "Don't know/Does not apply" responses. This resulted in a total of 4.80% of the data missing for the purposes of analysis, when both types were considered, with a median of 2.47% (range: 0-23.36%) per item.

2.2.4.2.iii Reliability

Intra-rater reliabilities (n = 54) were fair to excellent for MI-CAT(C)-v2 scale total, Subscales 1 and 2, and subcategories; inter-rater reliabilities (n = 32) were fair to good (Table 2.2.3). Amongst 29 households (owning 32 cats) for which inter-rater reliability was assessed, 14 secondary owners had more items with non-responses (range: 1-18 items) than the primary owners, while only five primary owners had more non-responses than secondary owners (range: 1-4 items). The numbers of response discrepancies other than non-responses varied widely between owner pairs (range: 1-18 different responses per pair); for the three households with two cats enrolled each, numbers of inconsistent responses between the primary and secondary owners were similar for both cats. Internal consistencies (n = 54) are shown in Table 2.2.4 (MI-CAT(C)-v2 scale total = 0.71-0.76). Inter-item correlations within each subscale were quite variable, ranging from -0.488 to 0.673 for Subscale 1, and from -0.443 to 0.908 for Subscale 2. Four items in Subscale 1 and 5 items in Subscale 2 had item-subscale total correlations < 0.20 on Days 0 and 15; these are indicated in italics in **Appendix A** (MI-CAT(C)-v2).

Table 2.2.3: Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – version 2 (MI-CAT(C)-v2) intra-rater and inter-rater reliabilities, based on assessments performed by the primary owner at Baseline (Days 0 and 15), and on concurrent assessment performed by the primary and a secondary owner (same day for each owner pair; study day selected based on owner pair convenience), respectively.

Scale component assessed		Intra-rater ICC-1 (95% CI) n = 54	Inter-rater ICC (95% CI) n = 32
Subscale 1	Agility	0.74 (0.59-0.84)	0.58 (0.30-0.77)
	Social/play/exploratory	0.70 (0.54-0.82)	0.58 (0.30-0.77)
	Self-maintenance	0.71 (0.55-0.82)	0.45 (0.12-0.68)
	Subscale 1 total	0.80 (0.69-0.88)	0.63 (0.37-0.80)
Subscale 2	Agility	0.69 (0.51-0.81)	0.54 (0.24-0.74)
	Self-maintenance	0.76 (0.62-0.85)	0.45 (0.13-0.69)
	Physical condition	0.59 (0.38-0.74)	0.61 (0.34-0.79)
	Subscale 2 total	0.72 (0.56-0.83)	0.60 (0.33-0.78)
MI-CAT(C)-v2 scale total		0.81 (0.69-0.88)	0.64 (0.39-0.81)

Legend: ICC = Intra-class correlation coefficient, based on a single rating, absolute agreement, one-way random effects model; 95% CI = 95% Confidence interval.

Table 2.2.4: Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – version 2 (MI-CAT(C)-v2) internal consistency at Baseline, *i.e.*, Days 0 and 15 (n = 54), expressed as Cronbach's alpha (95% confidence interval) for each subcategory and for each subscale (based on individual items), as well as for the scale total score (based on subcategory scores).

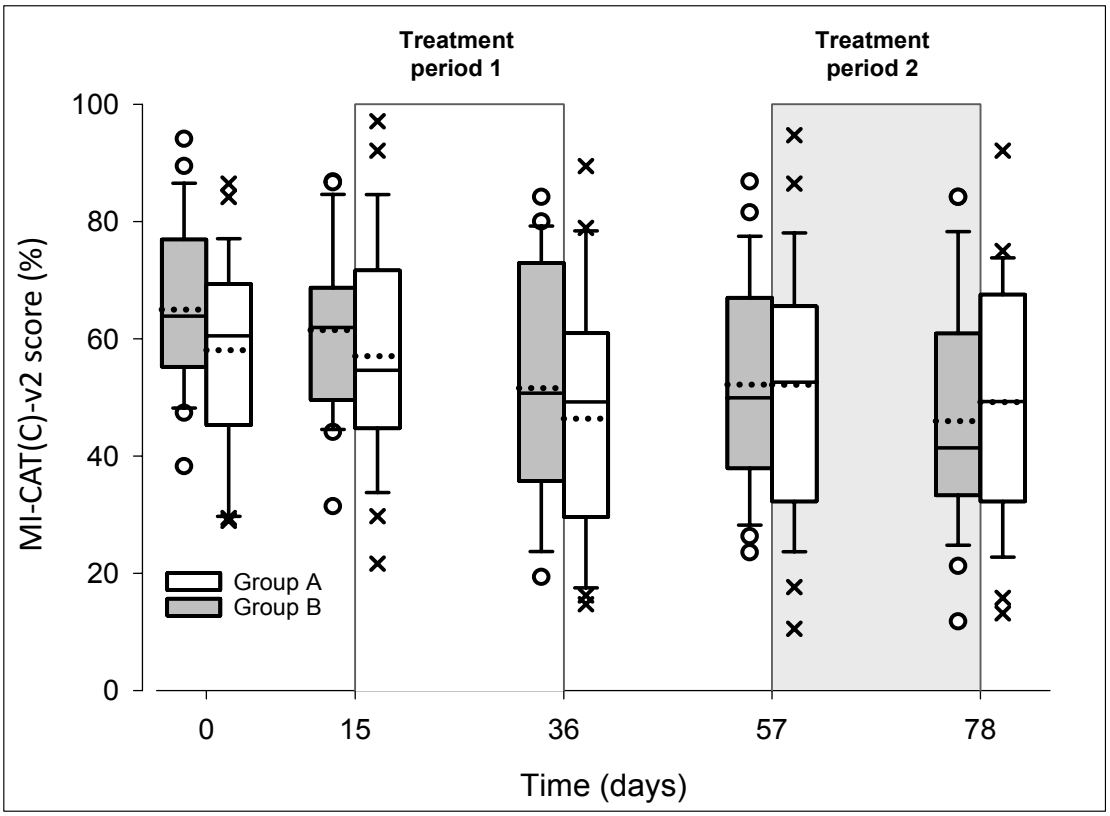
Scale component assessed		Cronbach's alpha	
		Day 0	Day 14
Subscale 1	Agility	0.46 (0.152-0.684)	0.57 (0.349-0.754)
	Social/play/exploratory	0.27 (-0.134-0.532)	0.46 (0.170-0.657)
	Self-maintenance	0.42 (0.073-0.647)	0.54 (0.277-0.725)
	Subscale 1 total	0.66 (0.483-0.811)	0.65 (0.484-0.821)
Subscale 2	Agility	0.63 (0.410-0.774)	0.69 (0.510-0.818)
	Self-maintenance	0.46 (0.187-0.680)	0.73 (0.568-0.825)
	Physical condition	-0.06 (-0.699-0.343)	0.29 (-0.119-0.571)
	Subscale 2 total	0.65 (0.416-0.786)	0.74 (0.578-0.851)
MI-CAT(C)-v2 scale total		0.76 (0.602-0.829)	0.71 (0.534-0.801)

2.2.4.2.iv Construct validity

2.2.4.2.iv.a Response to treatment

The distribution of MI-CAT(C)-v2 scores for both groups over time is presented in Figure 2.2.1. Analysis of the data recorded at 36 and 78 days of study revealed that the MI-CAT(C)-v2 was able to detect the effect of meloxicam in OA cats, with a mean score reduction of 17.56% compared to the reference level (see Table 2.2.5). Treatment period had no significant effect.

Figure 2.2.1: Group A and B Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – version 2 (MI-CAT(C)-v2) total scores over time.



Legend: Box plots of MI-CAT(C)-v2 total scores at each time-point for both Groups A and B. X and O symbols indicate values outside the 5% and 95% percentiles. Group A received meloxicam during Treatment period 1 (white), whereas Group B received it during Treatment period 2 (grey). Meloxicam treatment significantly ($P < 0.05$) influenced the MI-CAT(C)-v2 score at the end of the tested treatment periods (see text and Table 2.2.5 for details).

Table 2.2.5: Fixed effects of a generalized linear mixed-effect model of age (covariate), treatment, treatment period, and Client-Specific Outcome Measures (CSOM) scoring (at Baseline) for Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – version 2 (MI-CAT(C)-v2) total score, with sequence of treatment and cat nested within sequence as random factors.

Effect	Level	Estimate	Standard Error	Num DF	Den DF	F Value	Pr > F
Intercept		-1.6627	0.4525				
Age (days)		0.0003	0.0001	1	51	11.53	**0.001**
Treatment	Meloxicam	-0.1756	0.086	1	52	4.17	**0.046**
	Placebo	0	.				
Treatment period	1	0.0585	0.0859	1	52	0.46	0.499
	2	0	.				
CSOM at Baseline	High	0.5031	0.231	1	51	4.75	**0.034**
	Low	0	.				

Legend: *P*-values below the alpha threshold of 0.05 are indicated in bold. The reference (0) level was placebo treatment in Treatment period 2, with low client-specific outcome measures questionnaire (CSOM) score at Baseline. Num DF = Numerator degrees of freedom; Den DF = Denominator degrees of freedom; Pr = Probability.

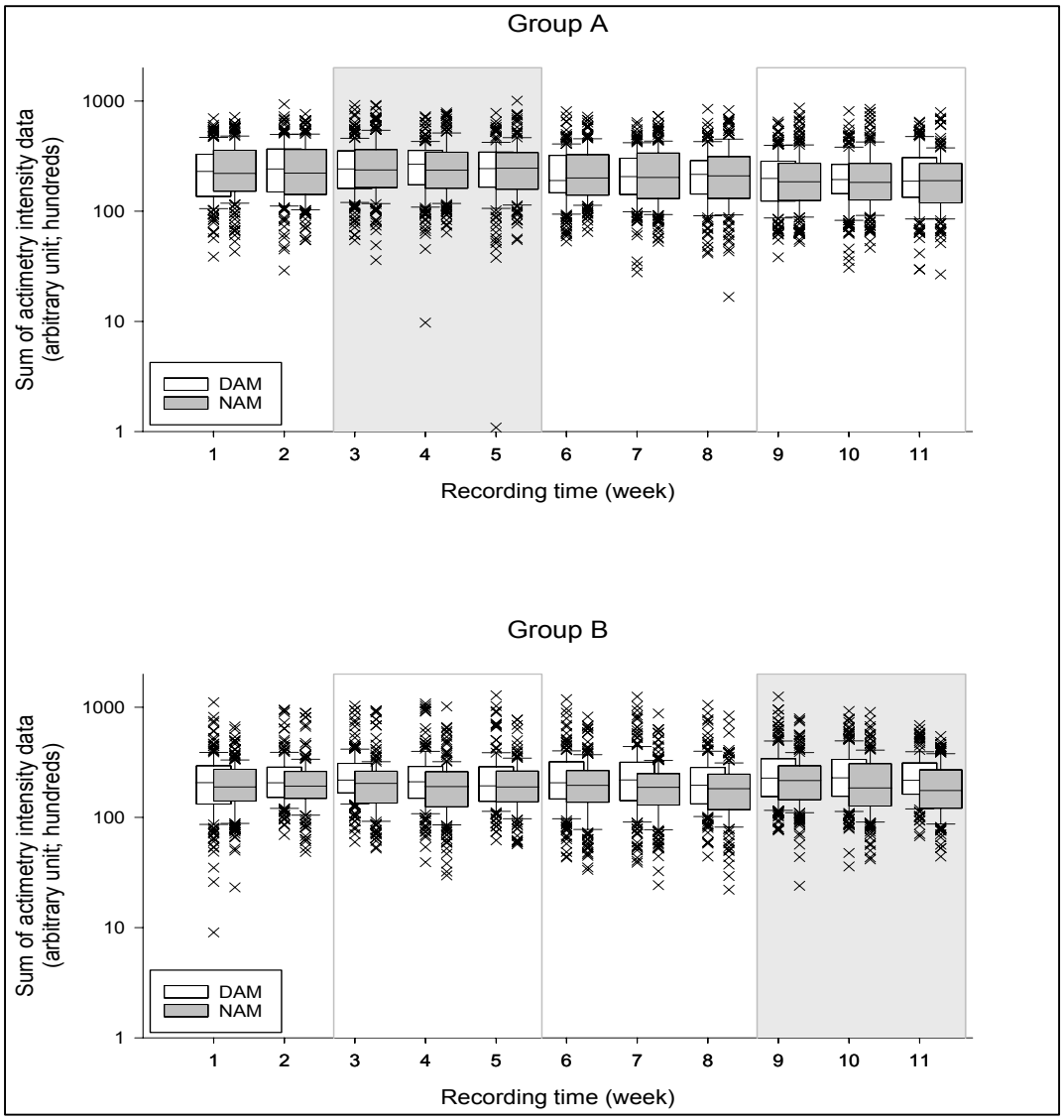
2.2.4.2.iv.b Convergent validity

Activity monitoring descriptive data, and the associated inferential analysis, are presented in Figure 2.2.2, and Table 2.2.6, respectively. Activity monitoring detected the effect of meloxicam in OA cats, with a mean increase in activity intensity of 23.83% compared to the reference level (see Table 2.2.6). Treatment period had a significant effect, but the interaction of treatment and treatment period did not. Night-time AM was lower than DAM overall, as well as for each treatment period. Compared with placebo, meloxicam

treatment produced a significant increase (Adjusted $P < 0.0001$) in both NAM (+15.1±1.76%) and DAM (+13.53±1.76%); the difference in the magnitude of the treatment effect, between the two, was not significant. The within-group difference (meloxicam *vs.* placebo) observed over the two treatment periods was significant for both Groups A and B (Adjusted $P < 0.0001$), but the between-groups comparison did not distinguish treatment from placebo ($P > 0.1866$).

The GLMM analysis indicated that the MI-CAT(C)-v2 score increased both with CSOM score and with age at Baseline (Table 2.2.5). Age and MI-CAT(C)-v2 were positively correlated ($Rho_P = 0.43$, $P = 0.0011$); the simple regression curve is shown in Figure 2.2.3-A (parameter estimate = 0.00008140, standard error (SE) = 0.00002345, $P = 0.0011$; $r^2 = 0.19$). The correlation between MI-CAT(C)-v2 and NAM was negative ($Rho_P = -0.36$, $P = 0.0074$); the simple regression curve is shown in Figure 2.2.3-B (parameter estimate = 0.12610, SE = 0.04524, $P = 0.0074$; $r^2 = 0.13$). MI-CAT(C)-v2 and DAM were also negatively correlated ($Rho_P = -0.27$, $P = 0.0443$). Age was negatively correlated with NAM ($Rho_P = -0.28$, $P = 0.0342$) and DAM (-0.30, $P = 0.0301$). Multiple linear regression analysis for MI-CAT(C)-v2 scores based on age and NAM (Table 2.2.7) produced an r^2 value of 0.22 and confirmed the significant relationships between MI-CAT(C)-v2 and both age and NAM; collinearity was not detected.

Figure 2.2.2: Actimetry intensity for Groups A and B, over time.



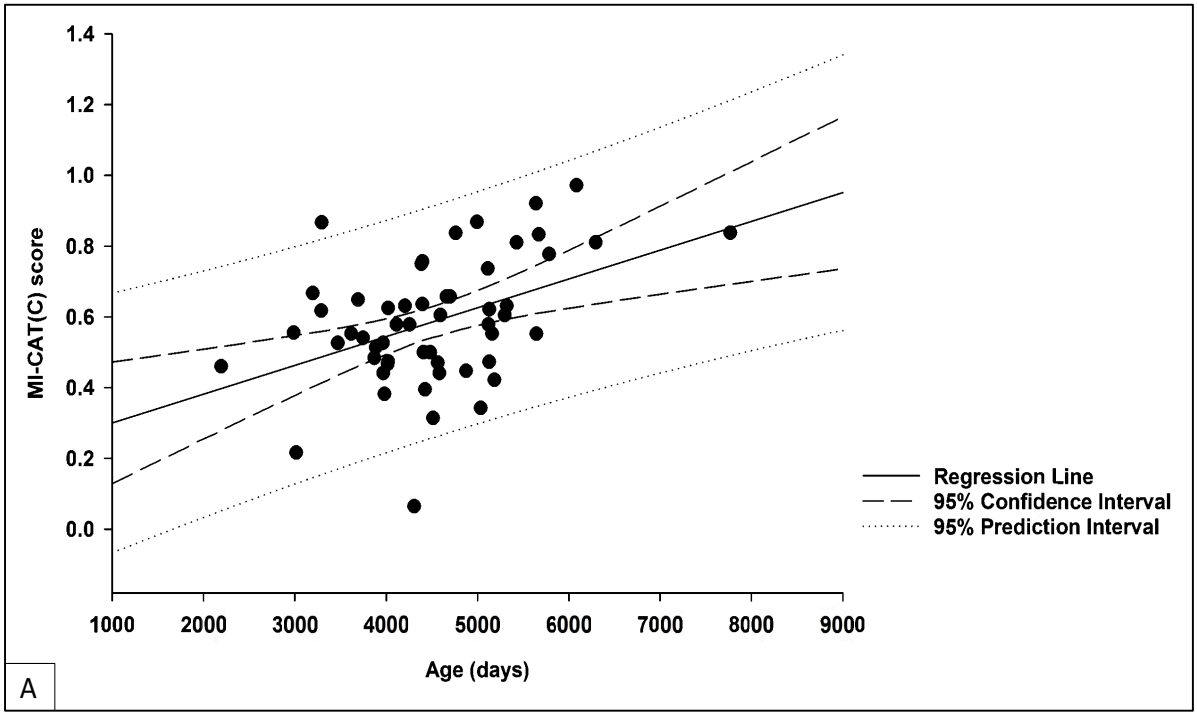
Legend: Box plots of activity monitoring data (AM) collected continuously for both Groups A and B. X symbols indicate values outside the 5% and 95% percentiles. The study periods subjected to statistical testing are highlighted with background boxes whose colours correspond to the type of treatment received during the period: white = placebo; grey = meloxicam. Meloxicam treatment significantly ($P < 0.0001$) increased the AM intensity, for both night-time AM (NAM) and daytime AM (DAM), during the treatment periods (see text and Table 6 for details).

Table 2.2.6: Fixed effects of a generalized linear mixed-effect model of treatment, treatment period, and night-day phase (night-time activity monitoring (NAM) and day-time activity monitoring (DAM)) for log-normally distributed 12-h sums of activity monitoring, with cat nested within treatment sequence as a random factor.

Effect	Level			Estimate	Standard Error	Num DF	Den DF	F Value	Pr > F
	Treatment	Period	Night-day phase						
Intercept				9.8569	0.0919				
Treatment	Meloxicam			0.2383	0.1322	1	3692	211.01	**<0.0001**
	Placebo			0	.				
Treatment period		1		0.136	0.1323	1	3692	25.45	**<0.0001**
		2		0	.				
Night-day phase			NAM	-0.0161	0.045	1	286.1	30.69	**<0.0001**
			DAM	0	.				
Treatment*treatment period	Meloxicam	1		-0.1815	0.2611	1	51.99	0.06	0.811
	Placebo	2		0	.				
Treatment*night-day phase	Meloxicam		NAM	-0.256	0.0627	1	288.6	0.15	0.7033
	Placebo		DAM	0	.				
Treatment period* night-day phase		1	NAM	-0.2355	0.063	1	288.6	0.05	0.8241
		2	DAM	0	.				
Treatment*treatment period*night-day phase	Meloxicam	1	NAM	0.4862	0.1013	1	286.1	23.02	**<0.0001**
	Placebo	2	DAM	0	.				

Legend: *P*-values below the alpha threshold of 0.05 are indicated in bold. The reference (0) level was placebo treatment in Treatment period 2, during daytime activity monitoring (DAM). NAM = night-time activity monitoring; Num DF = Numerator degrees of freedom; Den DF = Denominator degrees of freedom; Pr = Probability.

Figure 2.2.3: Simple regression analyses for Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – version 2 (MI-CAT(C)-v2) total score and age in days (Panel A; $r^2 = 0.19$), and for MI-CAT(C)-v2 total score and the log of night-time locomotor activity monitoring (NAM) (Panel B; $r^2 = 0.13$).



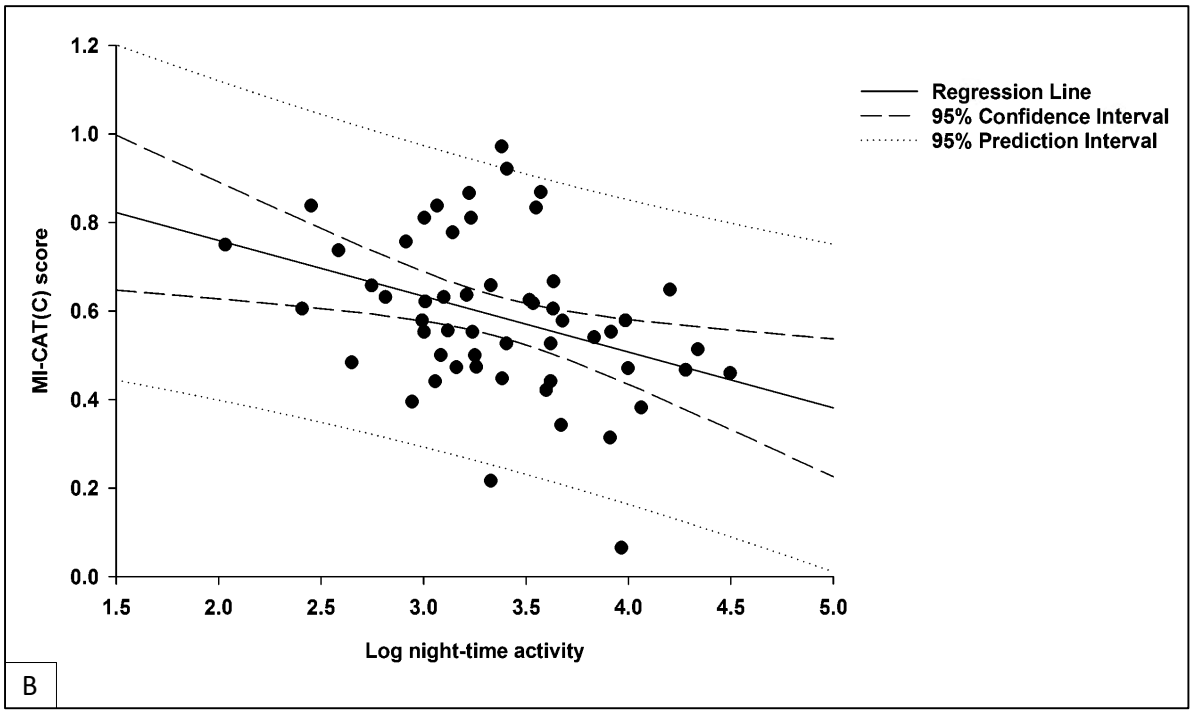


Table 2.2.7: Multiple regression analysis for Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – version 2 (MI-CAT(C)-v2) total scores, explained by age and log of night-time locomotor activity monitoring (NAM; $r^2 = 0.22$).

Variable	Parameter estimate	Standard error	*P*-value	Standardised estimate
Intercept	0.58378	0.20875	0.007	0
Log of NAM	-0.08980	0.04434	**0.048**	-0.25677
Age (days)	0.00006749	0.00002380	**0.006**	0.36726

Legend: *P*-values below the alpha threshold of 0.05 are indicated in bold.

2.2.4.2.iv.c Face validity

For 22 of 38 items, all owners indicated that they were clear/easy to understand. For nine items, one owner each responded that it was not clear; for four items, three to four owners responded in this way, and for three items, six to eight owners did so. Items with more than

one “not clear” response were either negatively worded (*e.g.* “My cat does not…”), or referred to Physical condition, and are indicated in bold font in **Appendix A** (MI-CAT(C)-v2).

2.2.5 Discussion

Feline OA pain remains difficult to evaluate, despite evidence that it responds to treatment (Bennett and Morton 2009, Clarke and Bennett 2006, Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Gruen, Thomson et al. 2016, Lascelles, Hansen et al. 2007, Lascelles, DePuy et al. 2010), making it challenging to evaluate novel therapies for efficacy, and disease progression and response to treatment in individual clinical patients. This study describes the development and preliminary validation and reliability testing of a feline OA pain scale. Expert evaluation supported the initial content of the scale; the pilot study (MI-CAT(C)-v1) permitted some refinements based on individual item analysis, but was limited by its laboratory context. For the MI-CAT(C)-v2, reliability was found to be good when completed by the primary owner. Convergence of scale scores with activity counts (and with age, as well as CSOM), and scale responsiveness to treatment with meloxicam, supported preliminary scale validity in this sample of cats.

Based on the expert review and pilot study, a number of modifications were made to the original scale, to produce the MI-CAT(C)-v2. Results of the content validation phase supported the representativeness and clarity of the scale. The pilot study (MI-CAT(C)-v1) suggested some problems, both with respect to internal structure of the scale (negative inter-item correlations), and also involving difficulty of assigning responses in some cases, lack of variability of responses, and poor reliability. However, because the pilot study involved only a small number of cats, and was conducted in a laboratory setting, these problems were interpreted relatively conservatively, and changes made to the MI-CAT(C)-v1 on the basis of the results were more limited than they might otherwise have been. The non-responses and poor reliability observed for certain items could have been due to differences between the laboratory setting and the relationship of the animal care attendants with the cats, from a home setting and the relationship of an owner with a pet cat. Alternatively, they could have reflected

poor item comprehension (as a comprehension analysis was not conducted) or other scale problems.

Various factors other than the scale itself may decrease reliability coefficients: the natural waxing and waning of OA, owner differences in observational capacities/relationship with the cat, and sample homogeneity (because reliability involves the ratio of inter-individual to total (*i.e.* including error) variability (Streiner and Norman 2008). In the clinical trial, the MI-CAT(C)-v2's intra-rater reliability (repeatability at an approximately two-week interval) was fair to excellent, and better than its inter-rater reliability. Owner pairs varied in their consistency; some secondary owners had much more difficulty answering scale items than did primary owners, implying less familiarity with the cat; no attempt was made to determine the secondary owners' degree of familiarity with their cats. Differences in owner-cat relationship may affect inter-rater error, *via* less familiar owners providing a high number of inaccurate or non-responses. Similar numbers of inconsistent responses for both cats, in households with two enrolled cats, could suggest that differences in owner familiarity on scale outcomes influenced the inter-rater reliability results. However, it is also possible that the primary owners consenting to participate in this study were unusually attentive to their cats, more so than cat owners in the general population (selection bias). The latter might be expected to produce an overestimation of scale performance. Though study owner age was similar to that recently reported for United States cat owners, the proportion of female respondents was higher (Saunders, Parast et al. 2017), possibly indicating that the sample was not representative of the cat-owning population at large. In any case, given a recommended minimum instrument reliability coefficient of 0.75 (Streiner and Norman 2008), we suggest that the owner most familiar with the cat complete the scale.

A low Cronbach's alpha suggests that different scale items are not measuring the same thing and may yield contradictory results; an excessively high statistic suggests redundancy in the scale. However, Cronbach's alpha also increases with the number of scale items. To partially account for this, the statistic was calculated for each of the subcategories and the subscales of the MI-CAT(C)-v2; additionally, the total scale internal consistency was assessed by calculating Cronbach's alpha based on the subcategory scores. In addition, item-subscale

correlations were assessed to identify items correlating poorly with the majority. This yielded an acceptable (between 0.70 and 0.90) MI-CAT(C)-v2 total internal consistency (Streiner and Norman 2008). The individual subcategory and subscale Cronbach's alphas were lower than 0.70, suggesting possible problems with internal consistency; based on Cronbach's alpha and 95% confidence intervals, this is a concern for most subcategories. In addition, inter-item correlations within each subscale were highly variable, indicating a lack of homogeneity, and nine items were determined to have low item-subscale total correlations. These findings suggest that further investigation of scale internal structure, and potentially removal or modification of specific items, are warranted. However, some study limitations likely influenced these results. First, the sample size was relatively small for estimating alpha. While there is not a clear consensus on sample size determination for scale validation studies, samples are commonly recommended either to have an absolute minimum number of 100 to 250 cases, or (typically for exploratory factor analysis) to have a subject to item ratio of 2 to 20 (Anthoine, Moret et al. 2014). The sample size for the clinical trial reported here was clearly low in relation to these recommendations; this was due to practical constraints, and is reflected in the broad confidence intervals observed for Cronbach's alphas and ICCs. Second, scale items were assigned to subcategories based on researcher judgement; factor analysis to determine the true loadings would been preferable (but was not possible due to sample size). Improper item groupings could have contributed to the low alpha results for some subcategories. Finally, all scale statistical analyses, including Cronbach's alpha, were calculated based on available item responses only; while the proportions of missing data were generally not high (with the primary exception of items relating to stair use, which was explained by the lack of access to stairs in some households), the lack of imputation could have introduced error into the results (Enders 2004).

Despite some questions about internal structure, the MI-CAT(C)-v2 was able to differentiate meloxicam treatment from placebo in this group of cats, which is promising, particularly given the randomised, blinded, crossover nature of the clinical trial. A large placebo effect complicating pain scale evaluation of analgesic efficacy has previously been reported in cats (Gruen, Griffith et al. 2014), and has been observed in other species. Potential causes include: normal disease variation, nonspecific treatment effects, regression to the mean,

and caregiver expectations or other respondent-dependent effects (Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Malek, Sample et al. 2012, Turner, Deyo et al. 1994). Ability to distinguish treatment from placebo is therefore an essential element of pain scale evaluation.

Meloxicam was associated with increased locomotor activity in client-owned OA cats, compared to placebo, similarly to what has been observed previously under laboratory conditions (Guillot, Moreau et al. 2013, Monteiro, Klinck et al. 2016). Increased locomotor activity with meloxicam treatment appears to be associated with relief of OA signs and improved animal comfort (Guillot, Moreau et al. 2013, Klinck, Gruen et al. 2017, Lascelles, Hansen et al. 2007, Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017), supporting the use of AM to validate the MI-CAT(C)-v2. Treatment effect was consistent in both periods, but the reference level over the treatment periods differed, explaining the significant treatment period effect and the absence of an effect of the interaction between treatment and treatment period. We previously reported NAM to be lower than DAM values, in laboratory OA cats (Guillot, Moreau et al. 2012), and also that NAM predicted OA treatment response better than did DAM, being less influenced by human activities (Guillot, Moreau et al. 2013, Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017). In the present clinical trial in client-owned OA cats, NAM was also lower than DAM, and NAM correlated most strongly with MI-CAT(C)-v2 scores. However, treatment effects did not differ significantly for NAM *vs.* DAM, possibly reflecting differences in the home *vs.* the laboratory environment. This is not altogether unexpected, given that owners are likely to be present and active in the evening, and may even interact with cats during the night, while little to no human interaction was available during the night in the laboratory studies. Meloxicam increased the average of NAM and DAM, compared to placebo, but the difference was less marked for Treatment period 1 (Group A) alone. The latter could call into question this aspect of construct validation, or could reflect a type II error in AM for Treatment period 1 (Group A) related to the limited sample size and the well-recognised inter-individual heterogeneity in AM outcomes (Gruen, Alfaro-Córdoba et al. 2017, Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013). Inter-cat variability could also explain the lack of a between-groups difference, despite the detectable within-group difference induced by the treatment. However, this could have been due in part to placebo effect (*e.g.*, increased owner attention producing a Hawthorne effect) beginning to influence

AM during the Baseline and decreasing the potential for improvement during Treatment period 1. Additionally, when taken together, the previously established relationship between OA pain and AM (Guillot, Moreau et al. 2012, Lascelles, Hansen et al. 2007), correlations between NAM and MI-CAT(C)-v2 scores, and significant NAM (and DAM) increase in response to meloxicam therapy, support construct (convergent) validity.

High impairment (based on CSOM score at Baseline) was associated with higher MI-CAT(C)-v2 scores. Age, which is distinct from OA but known to correlate positively with the presence and severity of radiographic OA (Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011), was also associated with MI-CAT(C)-v2 scores. While this supports the convergent validity of the scale, it is also possible that the scale is influenced by age-related factors other than OA. The collinearity analysis suggests age and NAM have distinct influences on MI-CAT(C)-v2 scores. This, and the scale's responsiveness to meloxicam, support that it is not simply detecting age-related activity decreases. A part of the MI-CAT(C)-v2 score variability is not explained by age and NAM, but neither is expected to mirror OA pain precisely.

It should be noted that no corrections were made for multiple comparisons in the assessment of convergent validity, only for the *post-hoc* analysis of AM. This was due to the preliminary nature of the study, the relatively small sample size, and the authors' determination that the cost of not detecting effects (a Type II error, potentially resulting in rejection of the scale or its components) would be greater than the cost of erroneously identifying an effect as significant. The impact of any Type I errors at this stage should be mitigated by further scale evaluation, which will in any case be needed prior to clinical use, as discussed further below.

Good owner comprehension for most items supports scale face validity. For the few items marked as "unclear" by three or more owners, it appears that negative wording or difficulty in understanding items relating to physical condition played a role. Such items may require revision. In addition, it should be noted that no thorough analysis of item comprehension has yet been performed for the scale. It is possible that some missing entries or "Don't know/Does not apply" responses were due to difficulty interpreting the affected items. There is a risk of

invalid results for any items with poor comprehension; hence, future research should include a comprehension analysis. Interestingly, there was some overlap between items with poorer comprehension, and those with item-subscale correlations < 0.20, again confirming the need for re-evaluation of said items.

An aspect of scale validity not assessed in this study was its ability to discriminate between OA and non-OA cats, as well as between different severities of OA; another is its responsiveness to different OA pain treatments. Although cats were screened for medical abnormalities other than OA, non-OA causes of pain responsive to meloxicam cannot be completely ruled out, and could have contributed to the improvements seen here, and altered the relationship between AM and the MI-CAT(C)-v2. Comparison of OA and non-OA cats would help to ascertain that the MI-CAT(C)-v2 truly measures OA pain, specifically. Unfortunately, recruiting non-OA cats is challenging due to high radiographic OA prevalence in the general, and especially in the aged, cat population (Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011). For clinical scale use, future research should also establish a threshold for determining OA *vs.* non-OA status, the degree of score change associated with the smallest improvement considered worthwhile (by owner or clinician), *i.e.* the minimum clinically important difference (Cook 2008, Copay, Subach et al. 2007), and the minimum detectable change, *i.e.* the minimum change expected to be beyond measurement error (Moreau, Pelletier et al. 2013).

2.2.6 Conclusions

The MI-CAT(C)-v2 was found to be reliable in this group of client-owned OA cats, when completed by the primary owner. Its ability to distinguish meloxicam treatment from placebo, and its correlations with AM, support its validity. However, limitations at this time include the need for further, larger scale studies. In particular, a comprehension analysis and an evaluation of internal structure should be performed, as well as testing to confirm scale ability to distinguish OA from non-OA cats and to guide its use in clinical decision-making, and refinements to minimise the influence of placebo and any non-OA effects.

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2.3 Preliminary Validation and Reliability Testing of the Montreal Instrument for Cat Arthritis Testing, for Use by Veterinarians, in a Colony of Laboratory Cats

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2.3.1 Simple summary

Feline osteoarthritis (OA) is challenging to diagnose. A pain scale was developed for use by veterinarians, in association with their physical examination, and tested for reliability and validity. The scale items were: Interaction with the examiner, Exploration of the room, Body Posture, Gait, Body Condition, condition of Coat and Claws, and abnormal Findings or Cat Reaction upon joint Palpation. Expert review supported the scale content. Two studies using laboratory-housed cats found the most promising results for Gait and Body Posture, in terms of distinguishing between OA and non-OA cats, repeatability of results, and correlations with objectively measured kinetics (weight-bearing).

2.3.2. Abstract

Subtle signs and conflicting physical and radiographic findings make feline osteoarthritis (OA) challenging to diagnose. A physical examination-based assessment was developed, consisting of eight items: Interaction, Exploration, Posture, Gait, Body Condition, Coat

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and Claws, (joint) Palpation–Findings, and Palpation–Cat Reaction. Content (experts) and face (veterinary students) validity were excellent. Construct validity, internal consistency, and intra- and inter-rater reliability were assessed *via* a pilot and main study, using laboratory-housed cats with and without OA. Gait distinguished OA status in the pilot ($p = 0.05$) study. In the main study, no scale item achieved statistically significant OA detection. Forelimb peak vertical ground reaction force (PVF) correlated inversely with Gait ($Rho_s = -0.38$ ($p = 0.03$) to -0.41 ($p = 0.02$)). Body Posture correlated with Gait, and inversely with forelimb PVF at two of three time points ($Rho_s = -0.38$ ($p = 0.03$) to -0.43 ($p = 0.01$)). Palpation (Findings, Cat Reaction) did not distinguish OA from non-OA cats. Palpation—Cat Reaction (Forelimbs) correlated inversely with forelimb PVF at two time points ($Rho_s = -0.41$ ($p = 0.02$) to -0.41 ($p = 0.01$)), but scores were highly variable, and poorly reliable. Gait and Posture require improved sensitivity, and Palpation should be interpreted cautiously, in diagnosing feline OA.

2.3.3. Introduction

Feline osteoarthritis (OA) has a high radiographic prevalence that increases with age (Lascelles, Henry III et al. 2010), and is increasingly recognized as an important cause of pain and loss of physical function (Bennett, Zainal Ariffin et al. 2012, Lascelles and Robertson 2010). Improvements have been reported in mobility (*e.g.*, jumping) and activity (Bennett and Morton 2009, Clarke and Bennett 2006, Giraudel, Gruet et al. 2010, King, King et al. 2016, Lascelles, Hansen et al. 2007, Lascelles, DePuy et al. 2010, Sul, Chase et al. 2014), lameness/stiffness (Gunew, Menrath et al. 2008, Lascelles, Hansen et al. 2007), mood (Bennett and Morton 2009, Giraudel, Gruet et al. 2010, Gunew, Menrath et al. 2008), and self-grooming (Bennett and Morton 2009) of cats with degenerative joint disease (DJD) including OA, in response to treatment with a non-steroidal anti-inflammatory drug, meloxicam (Bennett and Morton 2009, Clarke and Bennett 2006, Gunew, Menrath et al. 2008, Lascelles, Hansen et al. 2007, Sul, Chase et al. 2014) or robenacoxib (Giraudel, Gruet et al. 2010), or a therapeutic diet (Lascelles, DePuy et al. 2010). One study found that OA cats receiving dietary supplementation with long-chain omega-3 fatty acids showed improved mobility *vs.* placebo (Corbee, Barnier et al. 2013). However, the rate of diagnosis of this disease in the feline population appears to be low in relation to its radiographic prevalence (Lascelles 2010), and

physical examination findings (*e.g.*, lameness, abnormalities upon palpation) do not necessarily correlate with radiographic signs (Bennett, Zainal Ariffin et al. 2012, Clarke and Bennett 2006, Lascelles, Dong et al. 2012). Recent studies suggest that palpable abnormalities or pain are poorly sensitive for radiographic DJD in most joints (excepting the elbow, and lumbar and lumbosacral spine) (Lascelles, Dong et al. 2012), and the prevalence of radiographic signs in painful joints ranges from 33% (Lascelles, Hansen et al. 2007) in one study to 85% in another (Clarke and Bennett 2006). Palpable abnormalities other than pain (*e.g.*, decreased range of motion, joint thickening) are relatively uncommon in joints other than the elbow (Clarke and Bennett 2006, Lascelles, Hansen et al. 2007) and the hock (Clarke and Bennett 2006). One study found that palpation findings did not agree with historical signs of pain but did agree moderately with thermographic findings (Vainionpää, Raekallio et al. 2013). It appears that veterinarians rely heavily on owner-reported abnormalities to diagnose OA; diagnosis was rare in the absence of anamnestic signs in one study, and even cases with historical signs often lacked abnormalities upon palpation (Klinck, Frank et al. 2012).

Individual subject differences in pain experience and expression, and differences in examiner interpretive capabilities, are challenges in pain assessment. Objective measures such as peak vertical ground reaction force (PVF) (Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013, Moreau, Guillot et al. 2013, Schnabl and Bockstahler 2015), von Frey punctate tactile withdrawal threshold (VF) (Guillot, Moreau et al. 2012), telemetered locomotor activity monitoring (AM) (Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2007), thermographic imaging (Vainionpää, Raekallio et al. 2013), functional bio-imaging (Guillot, Chartrand et al. 2015), kinematics (Guillot, Gravel et al. 2015) and response to mechanical temporal summation (Guillot, Taylor et al. 2014) showed promise for detecting OA pain. However, these measures may not be feasible in clinical practice. In one study, AM did not distinguish OA from non-OA cats (due to high inter-individual variability), despite detection of treatment effects (Guillot, Moreau et al. 2012). Pain scales offer a relative objectivity and facilitate comparison within and between individuals (Robertson 2008). Recent research has sought to develop and validate owner pain scales for feline OA; examples include a client-specific outcome measure questionnaire (Lascelles, Hansen et al. 2007) and two standardized multi-item numerical rating scales (NRSs) (Benito, Depuy et al. 2013, Benito, Hansen et al.

2013, Bennett and Morton 2009, Zamprogno, Hansen et al. 2010). Scale validation is needed to confirm effective and consistent measurement of the condition of interest, in the target context. This process comprises multiple aspects addressed in separate experiments (Streiner and Norman 2008). "Content" validation assesses scale completeness and representativeness, generally *via* expert review; "face" validation comprises a similar assessment by a naïve population and also relates to acceptability of the scale (Crellin, Sullivan et al. 2007, Streiner and Norman 2008). "Criterion" validation uses a "gold standard" to evaluate scale performance, either at the same ("concurrent") or a later ("predictive") time (Streiner and Norman 2008). "Construct" validation evaluates how well the scale measures the condition of interest (*e.g.*, pain) when direct quantification using a gold standard is impossible (*e.g.*, for subjective experiences like pain or anxiety). It involves hypothesis testing (*e.g.*, between-groups comparison), and determination of convergence with related (*e.g.*, motor function), and divergence from distinct (*e.g.*, fear) constructs (Crellin, Sullivan et al. 2007, Streiner and Norman 2008). "Reliability" refers to degree of freedom from measurement error, which includes inter- (scoring consistency between evaluators) and intra-rater (scoring stability for one evaluator over time) reliability, and internal consistency (interrelatedness of scale items) (Crellin, Sullivan et al. 2007, Streiner and Norman 2008).

The objectives of this study were to develop a scale for use by veterinarians in conjunction with the physical examination, to perform content and face validation, and to assess both its ability to detect OA pain, and its reliability. We hypothesized that a standardized assessment combining examination room behavior, distance evaluation of posture and gait, and hands-on examination of body condition, and joint palpation and manipulation, could be used to detect clinical OA in cats.

2.3.4 Experimental section

2.3.4.1 Materials and methods

All study protocols were approved by the Institutional Animal Care and Use Committee (# Rech-1482). The guidelines of the Canadian Council on Animal Care were followed regarding cat care and handling.

2.3.4.2 Part I: scale development, and content and face validity

Preliminary content for the Montreal Instrument for Cat Arthritis Testing–Veterinarian (MI-CAT(V)) was developed based on a review of the literature and the authors' collective experience. The resulting eight items were each ranked on an NRS (ranging from zero to between two and six, where zero = normal). The proposed assessment was in two parts. The first was a distance observation including (1) Interactive Behavior with respect to the examiner, (2) Exploratory Behavior in the examination room, (3) Posture, and (4) Gait. The second was a hands-on evaluation of (5) Body Condition Score, (6) Coat and Claws (condition), (7) Joint Palpation (including manipulation) – Findings (*e.g.*, altered range of motion, crepitus, joint thickening, muscle atrophy), and (8) Joint Palpation (including manipulation) – Cat Reaction (*e.g.*, vocalization, withdrawal, tension, biting, scratching).

Content validity was tested *via* an internal (n = 3; MPK, DF, ET) and an external (n = 4; international experts in feline pain) evaluation. Each reviewer ranked scale items on their clarity, importance, and the appropriateness of response options (possible ranks of one to three; one = poor, two = fair, three = good), and commented on specific items, and general scale construction and content. Subsequent scale modifications included: (1) minor reordering of items evaluated *via* distance observation (Exploratory Behavior followed by Gait, Posture, then Interactive Behavior), (2) wording changes to improve clarity, and (3) expansion of the Posture, Coat and Claws, Palpation – Findings, and Palpation – Cat Reaction items. (See **Appendix B** for the MI-CAT(V)-v1).

Face validity consisted of a similar assessment by third-year veterinary students (n = 80). Respondent gender was noted, as well as whether they had ever: (1) owned a cat, (2) considered that cats could develop OA, and (3) assessed animal pain (acute or chronic). No subsequent scale modifications were made.

2.3.4.3 Part II: reliability assessment and construct validity

Evaluation of scale inter- and intra-rater reliability, internal consistency, and construct validity (distinction between OA and non-OA cats, concordance with functional and neurophysiological tests) was conducted in two phases, a pilot and a main study.

All cats were group-housed in dedicated, environmentally controlled rooms beginning four weeks prior to each study. A standard certified commercial diet (Hill's Prescription Diet® w/d® Feline, Hill's Pet Nutrition, Inc®, Mississauga, ON, Canada) was fed once daily, according to manufacturer recommendations, and water was supplied free choice. Cats could move freely about the rooms at all times, and enrichment was provided in the form of toys, windows, and climbing, perching and hiding areas.

Screening for OA consisted of complete physical examination, and digital radiography (DR; mediolateral and caudocranial views of stifle, coxofemoral, lumbosacral, sacroiliac, carpal and tarsal joints, and mediolateral views of shoulders and elbows). Cats were sedated for DR with intramuscular medetomidine (0.02 mg/kg; Domitor 1 mg/mL, Zoetis Canada, Kirkland, QC, Canada) and morphine (0.1 – 0.2 mg/kg; Morphine Sulfate Injection 10 mg/mL, Sandoz, Boucherville, QC, Canada). Inclusion criteria specific to OA cats were: (1) OA-related radiographic changes (osteophytosis, subchondral bone sclerosis, and/or joint surface remodeling) (Guillot, Moreau et al. 2012) with (2) orthopedic examination findings consistent with OA in the radiographically affected joint(s). Inclusion criteria for non-OA cats were: (1) the absence of OA-related radiographic changes in all joints assessed, and (2) a normal orthopedic examination. Cats could neither have received analgesic, anti-inflammatory or potential structuro-modulator (*e.g.*, glucosamine) medications during the three months prior to the study, nor have any clinically significant abnormalities other than OA on physical examination, complete blood count, blood chemistry (including T4), and urinalysis.

All MI-CAT(V) assessments were performed in a 1.5 × 3.9 m room with a 2-level (38 cm and 90 cm) examination table; cats were encouraged to move about and to jump up and down by calling, tossing treats or toys, petting, or brushing. Raters were blinded to cat OA status.

The pilot study included seven neutered, adult, domestic cats with both structural changes and orthopedic examination findings consistent with coxofemoral OA, and four with neither radiographic nor orthopedic examination findings consistent with OA in any joint. MI-CAT(V) evaluations were performed concurrently by two examiners (MK and PR) on Days zero, seven, 32 and 35; only MK (the reference observer) performed joint palpation and manipulation. Scale modifications based on the results included reorganization and expansion of the response options for Exploratory Behavior and Gait. Additionally, Body Condition, Coat and Claws, and Palpation – Findings were removed, and the response options for Palpation – Cat Reaction were simplified. (See **Appendix B**: MI-CAT(V)-v2).

For the main study, 120 neutered, adult domestic cats underwent OA screening. Thirty-eight met inclusion criteria and were retained: 32 OA (19 females and 13 males; mean (SD) of 8 (2.4) years), and six non-OA cats (three females and three males; 2.8 (1.4) years). Joints affected in the OA cats were, in order of decreasing prevalence: the hip (n = 21, 65.6%), shoulder (n = 12, 37.5%), tarsus (n = 12, 37.5%), stifle (n = 11, 34.4%) elbow (n = 9, 28.1%), and carpus (n = 4, 12.5%). Most cats had one affected joint in the forelimb, this number ranged from 0 to 4. In the hind limb, the median number of radiographically OA-affected joints was 2 with a range of 0 to 4. The two raters assessed a subset of the screened cats (n = 27) prior to the study start (day-34), each using the entirety of the revised scale, to evaluate its inter-rater reliability.

Prior to the study, cats were trained with food treats to traverse a pressure-sensing walkway, and were habituated to a von Frey test cage. One veterinarian (MPK) performed three, weekly scale assessments (days zero, seven and 14). Intra-rater reliabilities were calculated based on comparisons between pairs of days, and internal consistency and ability to detect OA were assessed for each day. Scale convergent (construct) validity was evaluated using the following tests: (1) PVF *via* a floor mat-based plantar force measurement system (Walkway® with Matscan® WE5 sensors, Tekscan, Boston, MA, USA), and (2) secondary punctate allodynia/hyperalgesia response *via* electronic VF withdrawal threshold measurements (Rigid Tip, 0.7 mm^2, 28 G; IITC Life Science, Woodland Hills, CA, USA), as previously described (Guillot, Moreau et al. 2013).

2.3.4.4 Statistical analyses

Intra- and inter-rater reliabilities were evaluated using Spearman's rank correlation (systematic biases) and a weighted Kappa for multiple categories (agreement) (Chen, Zaebst et al. 2005, Kundel and Polansky 2003). Interpretation of Kappa was based on that previously described, as follows: ≤0.00 = poor, 0.01 – 0.20 = slight, 0.21 – 0.40 = fair, 0.41 – 0.60 = moderate, 0.61 – 0.80 = substantial, 0.81 – 1.00 = almost perfect (Landis and Koch 1977). Spearman's rank correlations between individual scale item scores were used to evaluate internal consistency; they were also used to compare PVF and VF outcomes with scale scores. Interpretation of these correlations was as follows: 0 – 0.35 = weak, 0.36 – 0.70 = moderate, 0.71 – 1.00 = strong. The scale's ability to distinguish between OA and non-OA cats was tested using exact Wilcoxon-Mann-Whitney tests. All analyses were two-tailed with an α-level of 0.05, and analyses were performed using statistical software (SAS® system, version 9.2, SAS Institute Inc., Cary, NC, USA; JMP®, Version 9, SAS Institute Inc., 2010; Stata® Statistical Software, release 12, StataCorp LP. College Station, TX, USA, 2011).

2.3.5 Results

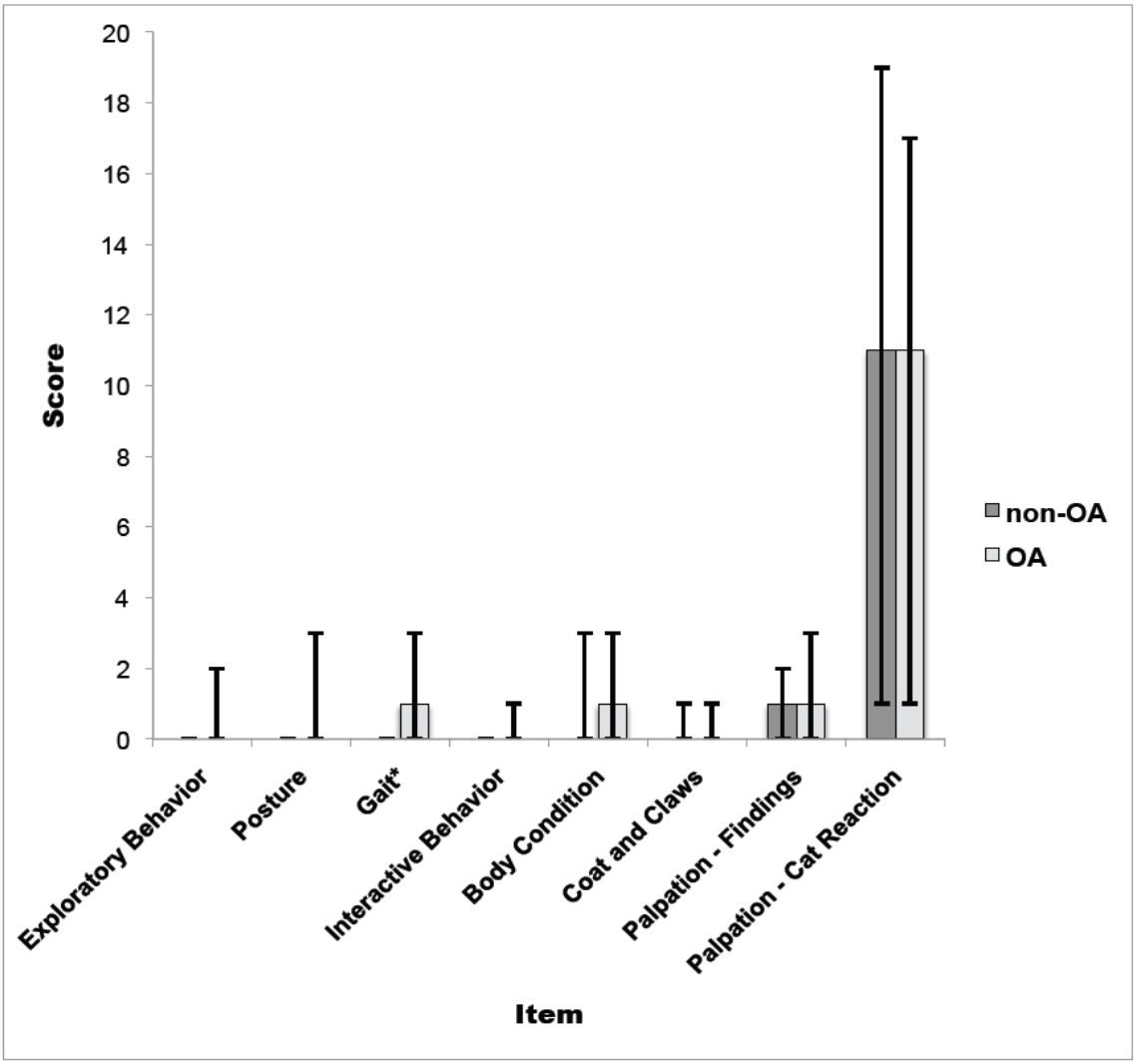
2.3.5.1 Part I: scale content and face validity

For the expert review, the median global item scores (*i.e.*, sum of clarity, importance, and appropriateness of response options scores) were either eight or nine out of nine for all items. A variety of comments on content and presentation were made, and these were incorporated into the MI-CAT(V)-v1 as described above.

Out of 80 students, 77 completed the review (96.3%). Many had owned cats (n = 62, 80.5%) or reported previously having evaluated pain in animals (n = 64, 83%); whether acute (n = 53, 68.8%) or chronic (n = 33, 42.9%). Forty-six (59.7%) had previously considered that cats could develop OA. All median global item scores were nine out of nine, except for Interactive Behavior (eight out of nine). Comments on content and presentation were varied, the most common being that individual temperament differences might affect the Exploratory

Behavior and Interactive Behavior items, and that Gait might not be evaluable in a clinic setting.

Figure 2.3.1: MI-CAT(V)-v1 scores by scale item for OA (n = 7) vs. non-OA (n = 4) cats (Pilot Study Day 0).



Legend: Medians are presented (error bars denote minimum and maximum). * p = 0.05.

For the pilot study (MI-CAT(V)-v1), all items on the scale, except Palpation – Cat Reaction, tended to be assessed at the low end of possible scores: scores of zero were frequent.

Only Gait distinguished between cats with and without OA ($p = 0.05$). (See Figure 2.3.1) Intra-rater reliability was fair to excellent for all items but Gait, Palpation – Findings, and Palpation – Cat Reaction. (See Table 2.3.1) Inter-rater reliability improved from the first (Day zero) to the second (Day 35) evaluation, when it was fair to excellent for all items. (See Table 2.3.2) There were significant correlations between: (1) Exploratory Behavior and Interactive Behavior (Spearman's Rho (Rho_S) = 0.74 ($p = 0.01$)), (2) Exploratory Behavior and Body Posture (Rho_S = 0.79 ($p < 0.001$)), (3) Body Posture and Gait ($Rho_S = 0.83$ ($p < 0.001$)), (4) Body Condition and Coat and Claw Condition ($Rho_S = 0.69$ ($p = 0.02$)), and (5) Palpation—Findings and Palpation—Cat Reaction ($Rho_S = 0.68$ ($p = 0.03$)).

Table 2.3.1: Intra-rater reliability for the Montreal instrument for cat arthritis testing–veterinarian – version 1 (MI-CAT(V)-v1) tested first at baseline over one week, and again over three days approximately one month later (n = 11 cats, 7 osteoarthritic (OA), 4 non-OA).

Scale Item	Days 0 and 7		Days 32 and 35	
	Kappa	Rho_s	Kappa	Rho_s
Exploratory Behavior	0.83	0.82 ($p < 0.0001$)	0.33	0.64 ($p = 0.03$)
Body Posture	0.64	0.60 ($p = 0.05$)	0.64	0.71 ($p = 0.01$)
Gait/Locomotion	−0.11	NS	−0.11	NE
Interactive Behavior	1.00	1.00 ($p < 0.0001$)	1.00	1.00 ($p < 0.0001$)
Body Condition	0.37	0.65 ($p = 0.03$)	0.37	1.00 ($p < 0.0001$)
Coat and Claws	0.80	0.82 ($p < 0.0001$)	0.80	NS
Palpation—Findings	0.18	NS	0.18	0.62 ($p = 0.04$)
Palpation—Cat Reaction	0.32	0.64 ($p = 0.04$)	0.32	0.64 ($p = 0.04$)

Legend: Rho_s = Spearman's rho; NS = not significant; NE = not evaluable.

Table 2.3.2: Inter-rater reliability (MI-CAT(V)-v1) for two observers tested on two occasions (n = 11 cats, 7 OA and 4 non-OA).

Scale Item	Day 0		Day 35	
	Kappa	Rho_s	Kappa	Rho_s
Exploratory Behavior	0.37	NS	0.84	0.85 ($p < 0.01$)
Body Posture	−0.41	NS	0.32	0.67 ($p = 0.02$)
Gait/Locomotion	0.10	NS	0.33	0.81 ($p < 0.01$)
Interactive Behavior	0.33	0.64 ($p = 0.03$)	0.62	0.67 ($p = 0.02$)
Body Condition	0.38	NS	0.52	0.59 ($p = 0.05$)
Coat and Claws	NE	NE	1.00	1.00 ($p < 0.01$)
Palpation – Cat Reaction	0.55	0.94 ($p < 0.01$)	0.68	0.86 ($p < 0.01$)

Legend: Rhos = Spearman's rho; NS = not significant; NE = not evaluable.

Four cats were withdrawn during the main study, three OA (one each due to vestibular syndrome, severe recurring diarrhea, and fear and aggression associated with handling), and one non-OA cat (due to unreliable responses to functional/neurophysiologic testing). Osteoarthritis was especially prevalent in the hindlimbs: 27 of 29 OA cats had at least one affected hindlimb joint. The coxofemoral (18/27 = 66.7%), tarsal (8/27 = 29.6%), and stifle (6/27 = 22.2%) joints were affected. The MI-CAT(V) evaluation took approximately 10 – 15 minutes per cat. Intra-rater reliability was best for Exploratory Behavior and Interactive Behavior, and acceptable for Body Posture with lower reliability for hind limb Posture. It was somewhat less good for Gait, and still less so for Reaction to Palpation. (See Table 2.3.3) Inter-rater reliability for the MI-CAT(V)-v2 was best for Interactive Behavior, Exploratory Behavior, and Body Posture (specifically, Posture of the hind limbs). The Gait assessment was poorly reproducible. Reliability results for Reaction to Palpation were heterogeneous, but acceptable for the hind limbs. (See Table 2.3.4) There were significant correlations at all time points between: (1) Exploratory Behavior and Interactive Behavior ($Rho_S = 0.42$ ($p = 0.01$) to 0.58 ($p = 0.0003$)), (2) Gait and Body Posture ($Rho_S = 0.65$ ($p < 0.0001$) to 0.73 ($p < 0.0001$)), and (3) Palpation – Cat Reaction and each of Exploratory Behavior ($Rho_S = 0.54$ ($p = 0.0009$) to

0.58 ($p = 0.0004$), Gait ($Rho_S = 0.77$ ($p < 0.0001$) to 0.83 ($p < 0.0001$)), and Body Posture ($Rho_S = 0.83$ ($p < 0.0001$) to 0.88 ($p < 0.0001$)).

No statistically significant differences were found between OA and non-OA cats for any scale items. Medians and ranges for individual items suggested non-significant trends for two, Gait and Body Posture, to yield higher (*i.e.*, worse) scores for cats with OA. Reaction to Palpation, particularly of the hind limbs, tended to yield higher scores for non-OA cats. (See Table 2.3.5) Exploratory Behavior and Interactive Behavior did not appear to be sensitive to OA status.

Von Frey and PVF distinguished between OA and non-OA cats, as reported elsewhere (Guillot, Moreau et al. 2013). Based on VF data distribution, an allodynia threshold was set at 40 g for the front paws and 50 g for the hind paws. These thresholds were determined based on the first quartile values of the OA cats, and no non-OA cat had such low values. Twenty-five percent of OA cats were allodynic *vs.* none of the non-OA cats. No associations were found between scale and VF assessments. There were moderate negative correlations between forelimb PVF and Gait at all time points ($Rho_S = -0.38$ ($p = 0.03$) to -0.41 ($p = 0.02$)). On days seven and 14, a moderate negative correlation was also found between Body Posture (Forelimbs) and forelimb PVF ($Rho_S = -0.38$ ($p = 0.03$) to -0.43 ($p = 0.01$)), and between Reaction to Palpation (Forelimbs) and forelimb PVF ($Rho_S = -0.41$ ($p = 0.02$) to -0.41 ($p = 0.01$)).

Table 2.3.3: Intra-rater reliability (MI-CAT(V)-v2) tested on three occasions, at one week intervals, (n = 34 cats, 29 OA and 5 non-OA).

Scale Item	Day 0–Day 7		Day 7–Day 14		Day 0–Day 14	
	Kappa	Rho_s	Kappa	Rho_s	Kappa	Rho_s
Exploratory Behavior	0.41	0.60 ($p = 0.0002$)	0.43	0.60 ($p = 0.0002$)	0.60	0.69 ($p < 0.0001$)
Body Posture						
Axial	0.58	0.72 ($p < 0.0001$)	0.69	0.86 ($p < 0.0001$)	0.84	0.85 ($p < 0.0001$)
Forelimbs	0.36	0.48 ($p = 0.0041$)	0.44	0.54 ($p = 0.001$)	0.40	0.40 ($p = 0.0176$)
Hind limbs	0.51	0.62 ($p < 0.0001$)	0.18	NS	0.57	0.74 ($p < 0.0001$)
Posture Total	0.40	0.69 ($p < 0.0001$)	0.40	0.57 ($p = 0.0004$)	0.54	0.78 ($p < 0.0001$)
Gait	0.33	0.68 ($p < 0.0001$)	0.38	0.45 ($p = 0.0072$)	0.28	0.54 ($p = 0.0011$)
Interactive Behavior	0.57	0.66 ($p < 0.0001$)	0.78	0.86 ($p < 0.0001$)	0.48	0.55 ($p = 0.0008$)
Reaction to Palpation						
Axial	NE	0.62 ($p < 0.0001$)	NE	0.62 ($p < 0.0001$)	NE	0.65 ($p < 0.0001$)
Forelimbs	0.26	0.72 ($p < 0.0001$)	0.36	0.52 ($p = 0.0018$)	0.38	0.56 ($p = 0.0006$)
Hind limbs	0.25	0.68 ($p < 0.0001$)	0.23	0.48 ($p = 0.004$)	0.44	0.75 ($p < 0.0001$)
Palpation Total	−0.02	0.72 ($p < 0.0001$)	0.00	0.63 ($p < 0.0001$)	NE	0.72 ($p < 0.0001$)

Legend: Agreement is assessed between each pair of evaluation days. Rho_s is Spearman's rho; NS = not significant; NE = not evaluable.

Table 2.3.4: Inter-rater reliability (MI-CAT(V)-v2) for two observers tested on one occasion (n = 27 cats, 22 OA and 5 non-OA).

Scale Item	Kappa	Rho_s
Exploratory Behavior	0.45	0.55 ($p < 0.0001$)
Body Posture		
Axial	0.31	NS
Forelimbs	−0.02	NS
Hind limbs	0.44	0.42 ($p = 0.0014$)
Posture Total	0.41	0.42 ($p = 0.0012$)
Gait	0.22	NS
Interactive Behavior	0.55	0.60 ($p < 0.0001$)
Reaction to Palpation		
Axial	0.24	0.29 ($p = 0.0306$)
Forelimbs	0.03	NS
Hind limbs	0.35	0.38 ($p = 0.0035$)
Palpation Total	0.21	NS

Legend: Rho_s is Spearman's rho; NS = not significant; NE = not evaluable.

Table 2.3.5: MI-CAT(V)-v2 scores by scale category for OA (n = 29) vs. non-OA (n = 5) cats, by evaluation time.

Scale Item	Day 0		Day 7		Day 14	
	OA	Non-OA	OA	Non-OA	OA	Non-OA
Exploratory Behavior	1 (1–6)	1 (0–5)	1 (0–6)	1 (0–5)	1 (0–5)	1 (0–5)
Body Posture						
Axial	0 (0–1)	0 (0)	0 (0–2)	0 (0–1)	0 (0–1)	0 (0)
Forelimbs	0 (0–1)	0 (0)	0 (0–2)	0 (0)	0 (0–1)	0 (0)
Hind limbs	1 (0–2)	0 (0–1)	0 (0–2)	0 (0)	0 (0–2)	0 (0–2)
Posture Total	1 (0–3)	0 (0–1)	1 (0–3)	0 (0–1)	1 (0–2)	0 (0–2)
Gait	3 (0–5)	1 (0–3)	3 (0–5)	0 (0–3)	3 (0–5)	2 (0–4)
Interactive Behavior	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–2)	0 (0–1)	0 (0–1)
Reaction to Palpation						
Axial	1 (0–5)	1 (0–3)	1 (0–5)	1 (0–2)	1.5 (0–4)	2 (0–3)
Forelimbs	2 (0–4)	3 (1–3)	2 (0–4)	3 (1–4)	2 (0–4)	3 (2–4)
Hind limbs	5 (0–8)	6 (3–7)	5 (1–9)	5 (2–6)	5 (2–7)	5 (4–6)
Palpation Total	5 (0–8)	6 (3–7)	5 (1–9)	5 (2–6)	5 (2–7)	5 (4–6)
Scale Total	9 (2–17)	11 (4–13)	8 (3–18)	10 (2–14)	9 (5–13)	10 (3–16)

Legend: Values are presented as median (range).

2.3.6 Discussion

The use of a combination of historical, orthopedic examination, performance test and radiographic findings has been recommended to reduce the uncertainty associated with feline OA diagnosis (Lascelles and Robertson 2010). However, there is clearly a mismatch between physical examination findings and radiographic signs of OA (Clarke and Bennett 2006, Lascelles, Dong et al. 2012, Lascelles, Hansen et al. 2007), as well as between historical and orthopedic examination findings (Corbee, Barnier et al. 2013, Klinck, Frank et al. 2012), and it is not evident just what performance tests may be most effective for detecting OA pain in clinical practice. Although owner pain scales for feline OA have recently been described (Benito, Depuy et al. 2013, Benito, Hansen et al. 2013, Bennett and Morton 2009, Lascelles,

Hansen et al. 2007, Zamprogno, Hansen et al. 2010), interpretation of physical examination findings in at-risk patients requires better understanding. This study attempted to determine the most reliable and valid examination procedures (including performance tests) for feline OA detection.

The MI-CAT(V) performed well in the initial naïve and expert reviews, supporting its content. Based on evaluation of reviewer comments, the existing literature, and the preliminary reliability and construct validity assessed *via* the pilot study, the items appearing least promising, Body Condition, Coat and Claws, and Palpation – Findings, were removed. The only item capable of detecting OA was Gait; none of the former distinguished OA from non-OA cats, nor were they correlated with Gait. Body Condition was variable in all cats and less reliable than anticipated; previous reports do not establish a clear relationship with musculoskeletal disease (Scarlett and Donoghue 1998, Slingerland, Hazewinkel et al. 2011). Other diseases and owner intervention may have a substantial effect on both Body Condition and Coat and Claws (*e.g.*, diet changes, brushing and claw-trimming). These physical aspects may therefore be more suitably assessed using an owner scale, and hence were not retained for further evaluation in the main study. Palpation – Findings other than pain were rare in the pilot study, consistent with other studies (Clarke and Bennett 2006, Slingerland, Hazewinkel et al. 2011); this item was therefore also eliminated prior to the main study. Of the retained items, Gait, Body Posture, Exploratory Behavior and Interactive Behavior demonstrated inter-item correlations in the pilot study, and Gait detected OA. The association between Exploratory Behavior and Interactive Behavior suggests convergence of these items, possibly evaluating the effects of chronic painful disease on cat temperament, whereas convergence of Body Posture and Gait would appear to be associated with biomechanical alterations and pain. Interestingly, Gait's intra-rater reliability was poor in the pilot study, but its inter-rater reliability was good at the second set of scale assessments. Given that inter-rater reliability of all items improved as the second veterinarian became more familiar with the scale (training effect), this may suggest that Gait had poor stability (*i.e.*, that it varied between assessment days). Gait was therefore revised in an attempt to improve its stability and sensitivity. Body Posture, Exploratory Behavior, and Interactive Behavior showed non-significant trends toward distinguishing OA from non-OA cats in the pilot study and were retained for further testing in

the main study. Palpation–Cat Reaction performed poorly in the pilot study, with respect to sensitivity to OA; however, given the clinical reliance on joint palpation as a diagnostic tool, it was revised and retained for further evaluation.

It was disappointing that no scale items distinguished OA from non-OA cats in the follow-up evaluation (MI-CAT(V)-v2), but, once again, Gait appeared the most promising for detecting OA signs. It may be that the presence of multiple affected joints/limbs makes postural and gait abnormalities more difficult to detect in feline OA. This apparent lack of sensitivity might be corrected in the future *via* alterations to the weights of these items. It is of note that these criteria, particularly Gait, are related to mobility/activity (Bennett and Morton 2009, Clarke and Bennett 2006, Giraudel, Gruet et al. 2010, King, King et al. 2016, Lascelles, Hansen et al. 2007, Lascelles, DePuy et al. 2010, Sul, Chase et al. 2014) and lameness/stiffness (Gunew, Menrath et al. 2008, Lascelles, Hansen et al. 2007), which have previously been described as sensitive to OA and responsive to anti-inflammatory treatment. However, reliability was generally good. Although both VF and PVF distinguished between OA and non-OA cats (Guillot, Moreau et al. 2013), we found correlations only between PVF and scale items, specifically, lower forelimb PVF was associated with increased (*i.e.*, abnormal) Gait, Body Posture (Forelimbs) and Palpation – Cat Reaction (Forelimbs) scores. The fact that most of the OA cats were affected in the hind limbs suggests that behavioral expression might be accentuated in the compensating (forelimb in the study) limbs. It appears that scale items did not detect hyperalgesia/allodynia, but may have detected functional changes (PVF), associated with OA. It should be noted that the presence of allodynia in 25% of OA cats, stable over time and unresponsive to meloxicam treatment (Guillot, Moreau et al. 2013), holds promise for neurophysiological pain assessment in the future. In the present study, none of the MI-CAT(V) scale items were constructed to detect this hypersensitivity. The item most expected to have done so would have been Reaction to Palpation, and this item was only associated with Exploratory Behavior, Gait and Body Posture. The relationship of the latter two suggests that they reflect some degree of biomechanical pain. Exploratory Behavior and Interactive Behavior, on the other hand, might be influenced more by the global temperament of the cat, reflecting neurophysiological changes and its individual experience of pain. It was particularly interesting that joint palpation and manipulation largely failed to

distinguish OA from non-OA cats. This was despite cat selection based in part on an apparently painful response to palpation, and did not change whether the assessment was subjective (*i.e.*, was there a painful response) or objective (*i.e.*, did the cat flinch, withdraw, vocalize, *etc.*). It is of note that, despite correlations with other scale items (Gait, Body Posture, and Interactive Behavior) and the correlation with forelimb PVF, Palpation – Cat Reaction actually had tendency for higher scores in non-OA than in OA cats, in contrast to a previous study's findings (Lascelles, Dong et al. 2012). This striking finding may explain the great difficulty in validating this type of response measure, and is potentially influenced by stimulus heterogeneity, subject temperament, and stress. On the basis of these results, the use of cat reaction to palpation cannot be recommended for clinical OA diagnosis. The authors are aware of no other studies of inter and intra-rater reliability of joint palpation findings in cats, and it may be that this method suffers due to poor reliability, or that it is simply not valid due to the effects of cat factors other than pain (*e.g.*, temperament). In any case, our findings call into question the emphasis to be placed on the findings of joint palpation and manipulation for evaluating feline OA. New developments addressing tactile hypersensitivity in OA cats are promising, such as the response to mechanical temporal summation (Guillot, Taylor et al. 2014). They now need to be translated into clinical assessment.

It should be noted that this research was conducted in a laboratory colony of cats, and that this may influence applicability of the results to the clinical context. These cats became familiar with the evaluators and the environment over the course of the study, and lacked some of the stressors present in clinical patients (*e.g.*, car travel, presence of unfamiliar animals). This may have influenced their behavior and facilitated assessment in the laboratory context. Cats may show inhibited or aggressive behavior in a clinical setting, due to fear, and, in the clinic, it is possible that this may be more of a problem in a population of client-owned cats. Future evaluation of the MI-CAT(V) will need to be performed to determine its feasibility in cats visiting a veterinary clinic. That being said, care was taken to make the environment similar to that of an examination room, the cats were not initially familiar with the room, scale procedure, or raters, and the evaluation itself was not so lengthy as to preempt its use in practice.

A frequent problem in the validation of pain scales is the lack of a true "gold standard" for measuring pain (Streiner and Norman 2008). We compared cats with both radiographic and physical examination findings consistent with OA, to cats with neither. Misclassification would have been possible given the difficulties inherent in the physical examination of feline OA, and the incomplete concordance between radiographic and clinical OA. However, this method was expected to yield a high index of suspicion for OA *vs.* non-OA status. The addition of concurrent functional (PVF) and neurophysiological (VF) evaluations related to OA (Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013, Moreau, Guillot et al. 2013) yielded a multifaceted approach to construct validation.

2.3.7 Conclusions

Based on the preliminary evaluation of the MI-CAT(V), a pain scale for use by veterinarians, it is concluded that the role of limb palpation in the diagnosis of feline clinical OA remains unclear; caution is urged in its interpretation. While body condition, and condition of coat and claws may be altered in OA, these should be assessed in light of the owner's interventions. The items assessing gait/locomotion, and possibly body posture, showed the most promise, but would benefit from further refinement to increase sensitivity and reliability in order to determine whether they can actually differentiate between cats with and without OA. They would then require evaluation for responsiveness to treatment, feasibility and usefulness in guiding treatment decisions, in client-owned animals. The importance of owner pain scales for use in feline OA is reaffirmed, given the uncertainties that remain regarding the interpretation of physical examination findings.

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2.3.9 Author contributions

Mary P. Klinck, Eric Troncy and Diane Frank developed the preliminary content and format for the MI-CAT(V). Eric Troncy, Martin Guillot, Mary P. Klinck and Pascale Rialland conceived of and designed the experiments. Mary P. Klinck and Pascale Rialland conducted the scale assessments of cats. Pascale Rialland, Martin Guillot, Maxim Moreau, Eric Troncy conducted all other assessments. Martin Guillot, Pascale Rialland and Mary P. Klinck analyzed the data. Mary P. Klinck edited the original manuscript and all co-authors revised it to produce the final version. Eric Troncy supervised the work at a whole.

2.3.10 Conflicts of interest

The authors declare no conflict of interest.

2.4 Refinement of the Montreal Instrument for Cat Arthritis Testing, for Use by Veterinarians: Detection of Naturally-Occurring Osteoarthritis in Laboratory Cats

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2.4.1 Abstract

2.4.1.1 Objectives

Feline osteoarthritis causes pain and disability. Detection and measurement is challenging, relying heavily on owner report. This study describes refinement of the Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians.

2.4.1.2 Methods

A video analysis of osteoarthritic (n = 6) and non-osteoarthritic (n = 4) cats facilitated expansion of scale items. Three successive therapeutic trials (using gabapentin, tramadol, and oral transmucosal meloxicam spray) in laboratory cats with and without natural osteoarthritis (n =12-20), permitted construct validation (assessments of disease status sensitivity and therapeutic responsiveness) and further scale refinements based on performance.

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2.4.1.3 Results

Scale osteoarthritis sensitivity improved from phase I to phase III; phase III scale total score (P = 0.0001), and 4/5 subcategories – body posture (P = 0.0006), gait (P = 0.0031), jumping (0.0824) and global distance examination (P = 0.0001) – detected osteoarthritic cats. Total score inter-rater (intra-class correlation coefficient (ICC) = 0.64-0.75), intra-rater (ICC = 0.90-0.91) and overall internal consistency (Cronbach's alpha = 0.85) reliability were good to excellent. Von Frey anesthesiometer-induced paw withdrawal threshold increased with gabapentin in phase I, in osteoarthritic cats ($P < 0.001$) but not in non-osteoarthritic cats (P = 0.075). Night-time activity increased during gabapentin treatment. Objective measures also detected tramadol and/or meloxicam treatment effects in osteoarthritic cats in phases II and III. There was some treatment responsiveness: in phase I, 3/10 subcategory scores improved ($P <$ 0.09) in treated osteoarthritic cats; in phase II, 3/8 subcategories; and in phase III, 1/5 subcategories improved ($P < 0.096$).

2.4.1.4 Conclusions and relevance

The revised scale detected naturally-occurring osteoarthritis, but not treatment effects, in laboratory cats, suggesting future potential for screening of at-risk cats. Further study is needed to confirm reliability, validity (disease sensitivity and treatment responsiveness) and clinical feasibility, as well as cut-off scores for osteoarthritic *vs.* non-osteoarthritic status, in client-owned cats.

2.4.2 Introduction

Osteoarthritis (OA) is common in cats; the radiographic prevalence of degenerative changes of the joints, including those associated with OA specifically, increases with age and is associated with pain and disability.(Bennett, Zainal Ariffin et al. 2012, Clarke and Bennett 2006, Lascelles 2010, Lascelles, Henry III et al. 2010) Clinical trials of nonsteroidal anti-inflammatory drugs (NSAIDs), meloxicam and robenacoxib, an anti-nerve growth factor (NGF) antibody, a therapeutic diet and dietary supplementation with long-chain omega-3 fatty acids have yielded improvements in mobility (*e.g.*, jumping) and activity (telemetric activity

monitoring (AM) or subjective assessment), lameness/stiffness, mood and grooming.(Bennett and Morton 2009, Clarke and Bennett 2006, Corbee, Barnier et al. 2013, Giraudel, Gruet et al. 2010, Gruen, Thomson et al. 2016, Guillot, Moreau et al. 2013, Gunew, Menrath et al. 2008, King, King et al. 2016, Lascelles, Hansen et al. 2007, Lascelles, DePuy et al. 2010, Sul, Chase et al. 2014)

Subtle and nonspecific OA signs in this species may be incorrectly attributed to mere aging, contributing to under-diagnosis of feline OA.(Bennett, Zainal Ariffin et al. 2012, Lascelles 2010) Clinical OA detection relies heavily on owner-reported historical abnormalities.(Bennett, Zainal Ariffin et al. 2012, Klinck, Frank et al. 2012) Lameness has been reported,(Clarke, Mellor et al. 2005, Klinck, Frank et al. 2012) but appears less prominent,(Bennett, Zainal Ariffin et al. 2012, Lascelles 2010) for example, than in the canine OA.(Belshaw, Asher et al. 2016) In addition, joint pain upon manipulation and palpable abnormalities may be poorly reliable,(Klinck, Rialland et al. 2015) and do not correlate highly either with radiographic or historical OA signs.(Corbee, Barnier et al. 2013, Guillot, Moreau et al. 2013, Klinck, Frank et al. 2012, Klinck, Rialland et al. 2015, Lascelles, Dong et al. 2012, Lascelles, Henry III et al. 2010, Vainionpää, Raekallio et al. 2013)

Difficulty detecting OA pain in cats impedes clinical case management, and novel drug testing. Recent studies describe objective and subjective (*i.e.*, pain scales) measures of feline OA pain and functional impairment. The former include AM,(Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2007) peak vertical ground reaction force (PVF),(Addison and Clements 2017, Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013, Monteiro, Klinck et al. 2017, Moreau, Guillot et al. 2013, Schnabl and Bockstahler 2015) thermographic imaging,(Vainionpää, Raekallio et al. 2013) functional bio-imaging,(Guillot, Chartrand et al. 2015) kinematics,(Guillot, Gravel et al. 2015) and measures of central sensitization, *e.g.*, von Frey punctate tactile withdrawal threshold (VF),(Addison and Clements 2017, Guillot, Moreau et al. 2012), response to mechanical temporal summation (RMTS),(Guillot, Taylor et al. 2014, Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017) and thermal sensitivity.(Addison and Clements 2017) These measures show promise but have practical limitations for clinical use, although a recent report supports the repeatability,

ability to detect OA status and feasibility of VF in naïve, client-owned cats.(Addison and Clements 2017)

Pain scales improve objectivity and facilitate comparisons within and between individuals.(Robertson 2008) They must be shown to measure the phenomenon of interest (*e.g.*, feline OA pain) reliably (*i.e.*, minimizing error), in the context of interest (*e.g.*, by the veterinarian, in the clinic).(Streiner and Norman 2008) Aspects of reliability include inter-rater (agreement between raters), intra-rater (repeatability over time given unchanged subject status) and internal consistency reliability (interrelatedness of scale components).(Crellin, Sullivan et al. 2007, Streiner and Norman 2008) Validation may include face (target user acceptability) and content (completeness/representativeness) validation, as well as criterion validation (*i.e.*, gold-standard comparison).(Streiner and Norman 2008) Construct validation for phenomena that are not directly measurable (*e.g.*, pain) comprises hypothesis testing (*e.g.*, therapeutic response, known group distinction such as OA/non-OA), and convergence with or divergence from, respectively, measures of related (*e.g.*, activity or weight-bearing) or unrelated (*e.g.*, temperament, sedation) phenomena.(Crellin, Sullivan et al. 2007, Streiner and Norman 2008) Reported feline OA owner scales include two standardized scales and a client-specific outcome measures questionnaire,(Benito, Depuy et al. 2013, Benito, Hansen et al. 2013, Bennett and Morton 2009, Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Lascelles, Hansen et al. 2007) but distinction between treatment and placebo effects remains challenging.(Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Gruen, Thomson et al. 2016) It is not known to what extent owner characteristics (*e.g.*, attentiveness) influence owner scale outcomes, and no other veterinary feline OA scales have been reported.

Development and preliminary validation for the Montreal Instrument for Cat Arthritis Testing, for Veterinarians (MI-CAT(V)), have been described.(Klinck, Rialland et al. 2015) This study's broad goal was refinement to improve scale OA sensitivity, and re-evaluation of validity and reliability in laboratory cats. Specific objectives included comparison of OA and non-OA cats to facilitate expansion of scale criteria, and subsequent comparisons of scale outcomes for 1) OA *vs.* non-OA cats; 2) OA cats before *vs.* after treatment; 3) treatment-associated changes in OA *vs.* non-OA cats; and 4) variation within and between veterinarians.

Refinement of the standardized MI-CAT(V) assessment was hypothesized to improve sensitivity to clinical OA in cats, while maintaining reliability.

2.4.3 Materials and methods

2.4.3.1 Ethics

The Institutional Animal Care and Use Committee approved the study (#Rech-1482, #Rech-1757). Cat care and handling adhered to the Canadian Council on Animal Care's guidelines.

2.4.3.2 Animals

Cats were group-housed in temperature- and humidity-controlled rooms containing environmental enrichment (access to windows, perches, covered and uncovered beds, scratching posts, and toys). Lights were turned on at 7 am, and turned off at 7 pm. A standard, certified, commercial diet (Hill's Prescription Diet w/d Feline; Hill's Pet Nutrition) was fed according to the manufacturer's directions; water was supplied ad libitum. Cats had no clinically significant abnormalities on complete blood count (CBC), serum chemistry (SC; including T4), and urinalysis, nor changes on general, neurologic, and orthopedic physical examinations other than those compatible with OA. Neither NSAID nor glucocorticoid administration was permitted for 4 or 8 weeks, respectively, preceding any study phase.

A board-certified veterinary radiologist analyzed digital radiographs of the appendicular joints, taken under sedation with intramuscular medetomidine (0.02 mg/kg Domitor 1 mg/mL; Zoetis Canada) and morphine (0.1–0.2 mg/kg Morphine Sulfate Injection 10 mg/mL; Sandoz).(Guillot, Moreau et al. 2012) Joints (views) assessed included the stifle (mediolateral and caudocranial), coxofemoral (ventrodorsal and lateral), carpal (dorsopalmar), tarsal (dorsoplantar), shoulders and elbows (mediolateral). Non-OA cats had neither radiographic nor orthopedic examination signs of OA at screening. Osteoarthritic cats had radiographic OA in at least one appendicular joint, and orthopedic examination abnormalities consistent with OA in the video analysis and phase I validation study (see below).

Subjective assessments were performed either in a 4.9 × 3.2 m (video analysis and phase I), a 4.1 x 4.0 m (phase II), or a 3.0 X 3.0 (phase III) room, containing either a two-level (38 cm and 90 cm) examination table and/or a chair with a seat height of 44 cm. Cats were encouraged to move about and to jump up and down by calling, tossing treats or toys, petting or brushing. All evaluators were blinded to cat OA and treatment status.

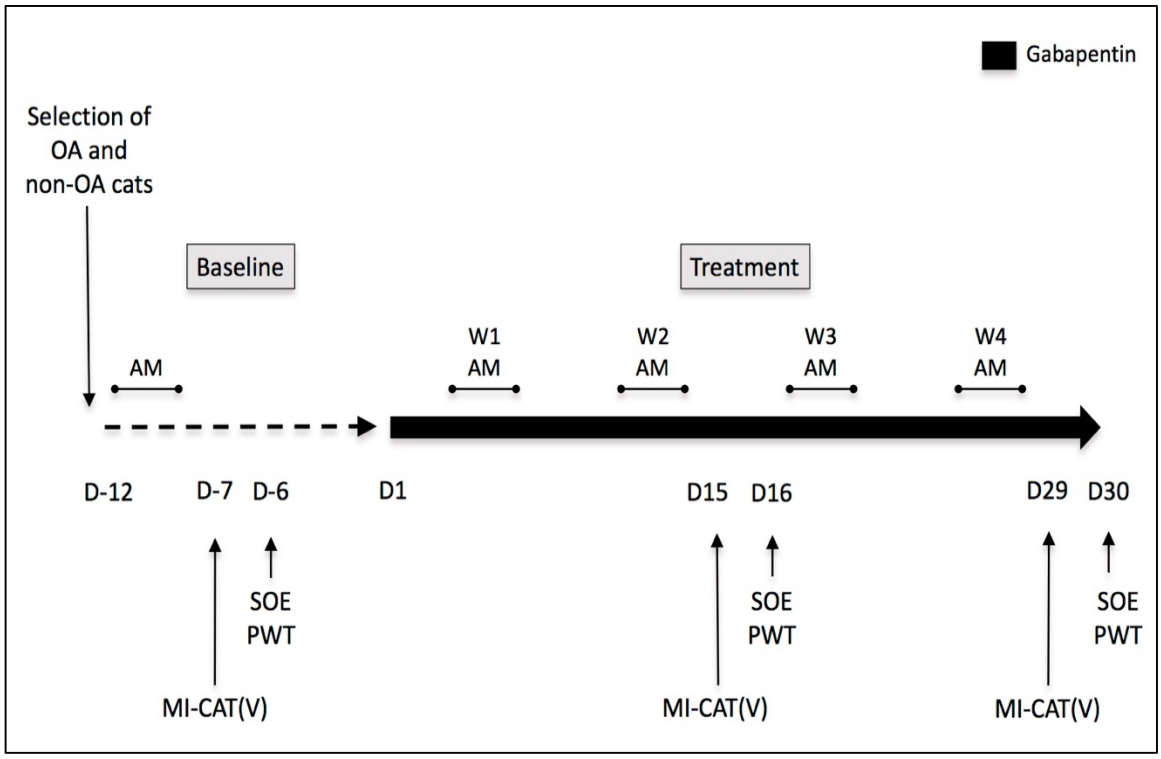
2.4.3.3 Video analysis

Six OA and four non-OA cats were videotaped individually while moving about an examination room. Four evaluators (one board-certified veterinary surgeon, two board-certified veterinary behaviorists, one veterinary student) reviewed the videos under blinded conditions. Evaluators were simply asked in an open-ended manner to identify criteria, particularly pertaining to posture, gait, and willingness to move about, which varied between cats. These were used to formulate new items and detailed evaluation procedure instructions, and to reformat response options. The MI-CAT(V)-v3 (**Appendix C**) included 67 ordinal scale items in 10 subcategories: body posture – back (BP-B); body posture – forelimbs (BP-F); body posture – hind limbs (BP-H); gait – general; gait – forelimbs (G-F); gait – hind limbs (G-H); willingness and ease of horizontal movements (WEHM); standing up on hindfeet to investigate a higher surface (SUHF); jumping; other behaviors (OB).

2.4.3.4 Validation phase I

Sensitivity to OA and responsiveness to treatment were assessed *via* a therapeutic trial. A power calculation based on mean VF anesthesiometer-induced paw withdrawal threshold (PWT; power = 0.80, α = 0.05, one-sided PWT increase from 60 g to 80 g) yielded a minimum sample size of six OA cats. Gabapentin (gabapentin 100 mg/mL oral suspension, Gentès & Bolduc Pharmaciens) was administered to seven OA and five non-OA cats, at a dose of 10 mg/kg orally three times daily, for 30 days. Additional inclusion criteria were the presence (OA) or absence (non-OA) of allodynia, as described below. See Figure 2.4.1 for study design.

Figure 2.4.1: Validation phase I trial design.



Legend: OA = osteoarthritic; D = day; W = week; AM = locomotor activity monitoring; SOE = surgeon's orthopedic evaluation; PWT = von Frey anesthesiometer-induced paw withdrawal threshold; MI-CAT(V) = Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians.

The MI-CAT(V)-v3, a surgeon's orthopedic evaluation (SOE) (**Appendix C**), and PWT assessments were completed once at baseline (day (D)-7/-6) and twice during the treatment period (D15/16 and D29/30). Cats wore AM sensors from D-12 to D30; night-time AM (NAM) data (6 pm to 5:58 am) collected from Friday to Monday were considered for analysis,(Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013) for D-12 to D-9 (baseline), D3-D6 (week (W)1), D10-D13 (W2), D17-D20 (W3), and D24-D27 (W4).

2.4.3.4.i Von Frey-anesthesiometer-induced paw withdrawal threshold

Allodynic status was determined based on measurements of perpendicular pressure on the palmar/plantar aspect of the paw in standing cats, using a mechanical VF polypropylene probe (Rigid Tip 0.7 mm^2 28 G; IITC Life Science).(Guillot, Moreau et al. 2013) Peak force was measured twice per paw, 60 s apart, with stimulus cessation upon paw withdrawal/behavioral signs of pain. Data under 2 g were discarded, and a maximal cut-off of 200 g was applied. The allodynia threshold was 50 g for front- and hind-paws, based on the data distribution. All OA cats had at least one paw with a mean threshold < 50 g and duplicate measurements < 60 g. Non-OA cats had single paw mean thresholds ≥ 60 g, and no duplicate measurements for any paw < 80 g. The PWT was calculated by averaging available measurements (n = 8) for each cat, at each time point.

2.4.3.4.ii Activity monitoring

Collar-mounted accelerometer-based activity sensors (ActiWatch-mini; Minimitter, Bio-Lynx Scientific Equipment) made counts every 2 mins; numeric amplitude (0 to infinite, no unit) was based on intensity.(Guillot, Moreau et al. 2013)

2.4.3.4.iii Subjective measures

A board-certified veterinary behaviorist (MK) completed the MI-CAT(V)-v3. A board-certified veterinary surgeon (BL) performed the SOE, consisting of palpation/manipulation of each axial segment and appendicular joint for pain (numerical rating scale (NRS), 0-10) or physical abnormalities (heat, edema, thickening, effusion, instability, crepitus, reduced range of motion; scored as present/absent) and distance observation for lameness (NRS, 0-10; based on evaluation of gait, posture, and ease of movements, such as when rising from a recumbent position).

2.4.3.4.iv Scale revisions

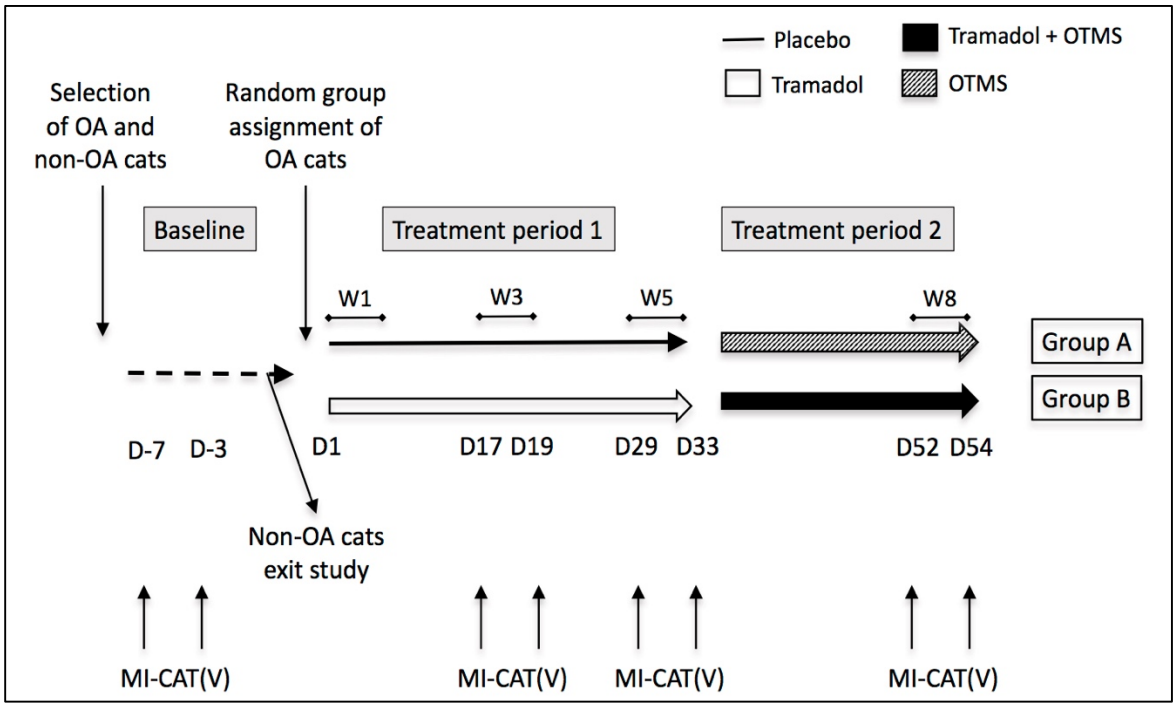
Subsection and individual item (data not shown) performance guided scale modifications. A global distance examination (GDE; lameness) was added. Next, a

veterinarian naïve to the scale (BM) evaluated the MI-CAT(V) for clarity and ease of use. Altered scoring, minor rewording, reordering and removal of several items, and condensing of the gait subcategories into one, and the jumping and SUHF subcategories into one, produced the MI-CAT(V)-v4 (**Appendix C**), with 44 items in eight subcategories.

2.4.3.5 Validation phase II

Scale internal consistency and inter- and intra-rater reliability, and detection of OA status and response to treatment (construct validity), were assessed *via* a therapeutic trial, details of which have been previously described in the context of a study assessing treatment effects *via* objective measures (PVF, NAM, RMTS).(Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017) See Figure 2.4.2 for the trial design. A power calculation based on Phase I MI-CAT(V) data (power = 0.80, α = 0.05, MI-CAT(V) one-sided total score decrease from 0.25 to 0.18) yielded a minimum sample size of 13 OA cats. Fifteen OA and five non-OA cats participated at baseline (D-7 to -1). Osteoarthritic cats were then randomly assigned to two treatment groups, with observers blinded to treatments and OA status. In period 1 (D1-D33), group A (n = 7) received placebo (15 mg corn starch) and group B (n = 8) received identically appearing tramadol (3 mg/kg Tramadol HCl; Gentès & Bolduc Pharmaciens), orally twice daily.(Monteiro, Klinck et al. 2017) In period 2 (D33-D54), meloxicam oral transmucosal spray (OTMS; approximately 0.05 mg/kg OroCAM Oral TransMucosal Spray, 0.25 mg/spray; Abbott Animal Health) was also given (Group A: placebo and OTMS; Group B: tramadol and OTMS).(Monteiro, Klinck et al. 2016)

Figure 2.4.2: Validation phase II trial design.



Legend: OA = osteoarthritic; D = day; W = week; MI-CAT(V) = Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians; OTMS = meloxicam oral transmucosal spray.

Two evaluators, one familiar with (MK), and one new to the MI-CAT(V)-v4 (BM), completed it at D-7, D-3 (baseline), D17, D19 (W3), D29, D33 (W5), D52 and D54 (W8). Naïve evaluator training involved reviewing video examples (n = 3 cats) of scale criteria at different score levels and practicing the MI-CAT(V)-v4 evaluation procedure with two of her own cats.

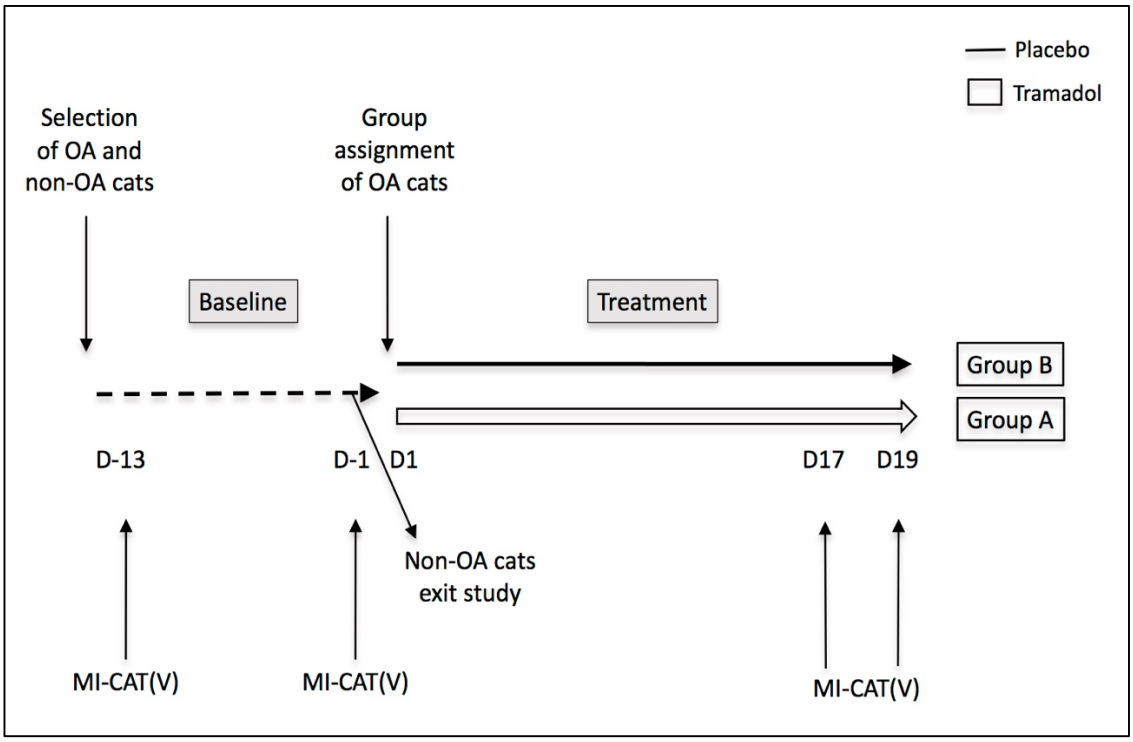
2.4.3.5.i Scale revisions

Subsection and individual item (data not shown) performance guided scale modifications, comprising minor changes to instructions, removal of several items (including the OB subcategory), condensing of body posture subcategories into one, and scoring changes. The MI-CAT(V)-v5 had 25 items in five subcategories (**Appendix C**).

2.4.3.6 Validation phase III

Scale internal consistency and inter- and intra-rater reliability, and detection of OA status and response to treatment, were assessed *via* a therapeutic trial, details of which have been previously described in the context of a study assessing treatment effects *via* objective measures (PVF, NAM, RMTS).(Monteiro, Klinck et al. 2017) See Figure 2.4.3 for trial design. Cats (n = 13 OA, n = 6 non-OA) had participated in phase II with the following changes: two OA cats were unavailable,(Monteiro, Klinck et al. 2017) and an additional non-OA cat was included. Osteoarthritic and non-OA cats participated at baseline (D-13 to D-1). Osteoarthritic cats received tramadol or placebo (as described above), based on their phase II – period 1 treatment group assignment (crossover). Those having received placebo in phase II – period 1 received tramadol in phase III, as described above, and vice-versa; group A (n = 6) received tramadol and group B (n = 7) received placebo, from D1 to D19. MI-CAT(V)-v5 assessments were performed at D-13, D-1, D17 and D19, by two veterinarians (MK and BM), with observers blinded to treatments and OA status.

Figure 2.4.3: Validation phase III trial design.



Legend: OA = osteoarthritic; D = day; MI-CAT(V) = Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians.

2.4.3.7 Statistical methods

All analyses were conducted with statistical software (SAS system, version 9.3 (SAS Institute), and SPSS Statistics for Macintosh, version 20 or 24 (IBM)). Data were assessed for normality using the Shapiro-Wilk test. Model covariance structures were based on information criteria. An exploratory α level was set at 0.10 for subjective measures, with no corrections for multiple comparisons, to maximize the chances of significant results in a comparative pilot study setting of MI-CAT(V) and SOE with such small sample sizes. It is acceptable to set a higher α value, when the goal of the study is to find an effect that could lead to a promising scientific discovery. This allows us not only to increase the power and consequently decrease the risk of a type II error, but also increases the chances of making a type I error (*i.e.*, saying

there is a difference when there is not); $\alpha = 0.05$ in all other instances. Analyses were two-tailed, except as noted below.

2.4.3.7.i Phase I

Analyses were one-tailed for treatment effects in OA cats (H_0 = treatment does not improve outcomes; H_1 = treatment improves outcomes).(Murphy 2017) Treatment effect on PWT was analyzed *via* a linear mixed model for repeated measures (fixed effects: day, OA group and their interaction; compound symmetry covariance structure). For each NAM evaluation period (3 days x 12 h), every 10 successive NAM recordings (*i.e.*, over 20 mins) were averaged from 6 pm to 5:58 am, yielding 108 average NAM values for each period (baseline, W1, W2, W3, W4) for each cat. Log-plus-one-transformed means were analyzed *via* a generalized estimating equation model (fixed effects: time, OA status and their interaction; repeated measurements: time, day (Friday-Monday) and recordings; exchangeable covariance structure). Total MI-CAT(V) and subsection 1-10 scores were sums of individual item scores. Total SOE score was the sum of all pain and other physical abnormality scores (maximum possible = 334); this was also subdivided into total axial score (maximum possible = 52) and total individual limb scores (maximum possible for each = 68). Total pain score was the sum of axial segment and appendicular joint pain scores (maximum possible = 210); total palpation score was the sum of pain and physical abnormalities scores for all axial and appendicular joints (maximum possible = 324). Long bone scores were used only to rule out orthopedic abnormalities unassociated with the joints. Non-parametric Wilcoxon Rank Sum tests (baseline; OA *vs.* non-OA) and Wilcoxon Signed Rank tests (pre- *vs.* post-treatment) were used for MI-CAT(V) and SOE score analyses.

2.4.3.7.ii Phases II and III

The MI-CAT(V)-v4/v5 total score was a percentage of the maximum possible score (**Appendix C**). Exact Wilcoxon-Mann-Whitney tests evaluated sensitivity to OA status (phase II D-7; phase III D-13). Treatment effect was analyzed *via* a generalized linear mixed model (fixed effects: treatment group, week and their interaction; random effect: cat; compound symmetry covariance structure; phase II – period 1: baseline *vs.* W3 and W5) or paired t-tests

(phase II – period 2: baseline *vs*. W8; phase III: baseline *vs*. D19). Internal consistency was assessed using Cronbach's α (phase II: D-7, D-3; phase III: D-1), based on the experienced evaluator's (MK) scores. Average measures for two-way random intra-class correlation coefficients assessing for consistency are reported for inter-rater (phase II: D-7, D52; phase III: D-13, D17) and intra-rater (phase II: D-7 and D-3, D52 and D54; phase III: D-13 and D-1, D17 and D19) reliability. Interpretation was as follows: < 0.40 = poor; 0.40-0.59 = fair; 0.60-0.74 = good; 0.75-1.00 = excellent.(Cicchetti and Sparrow 1981)

2.4.4 Results

2.4.4.1 Phase I

Mean age was 5.98 years (range 2.3-12.4 years) for non-OA cats and 8.27 years (range 4.3-12.4 years) for OA cats. No serious adverse events attributable to gabapentin were noted on clinical, CBC and SC analyses. No long bone abnormalities were detected on the SOE. See Table 2.4.1a for OA *vs.* non-OA MI-CAT(V) results. Only WEHM detected OA status (P = 0.048); no aspect of the SOE did so ($P \geq 0.537$). Table 2.4.1b presents MI-CAT(V)-v3 results for OA cats at baseline *vs.* D29. Following treatment, OA cat scores decreased significantly (improved) for BP-B (P = 0.09), G-F (P = 0.055) and SUHF (P = 0.0785); G-H scores increased (P = 0.09). For the SOE, only the distance score improved (P = 0.055). Treatment did not affect MI-CAT(V) and SOE scores for non-OA cats.

Osteoarthritic status (P = 0.012), time (P = 0.001) and their interaction (P = 0.041) significantly affected PWT. Figure 2.4.4 shows OA and non-OA mean PWT over time; the univariate effect of time was significant for OA ($P < 0.001$) but not non-OA cats (P = 0.075).

Table 2.4.1a: Phase I baseline comparison of Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 3 (MI-CAT(V)-v3) scores, expressed as median (range), for osteoarthritic (OA) and non-OA cats.

Scale component assessed	OA cats n = 7	Non-OA cats n = 5	*P*-value
1. Body posture – back	0 (0-3)	1 (0-3)	0.530
2. Body posture – forelimbs	1 (0-3)	1 (0-1)	1.000
3. Body posture – hind limbs	3 (0-8)	1 (1-3)	0.268
4. Gait – general	1 (0-4)	2 (0-5)	0.432
5. Gait – forelimbs	0 (0-3)	1 (0-5)	0.343
6. Gait – hind limbs	1 (0-2)	0 (0-4)	0.639
7. Willingness and ease of horizontal movements	4 (0-8)	0 (0-3)	0.048*
8. Standing up on hind feet to investigate a higher surface	0 (0-4)	0 (0-0)	0.432
9. Jumping	5 (1-7)	6 (2-7)	0.202
10. Other behaviors	0 (0-1)	0 (0-2)	0.876
Total MI-CAT(V)-v3 score	13 (6-28)	16 (7-26)	0.755

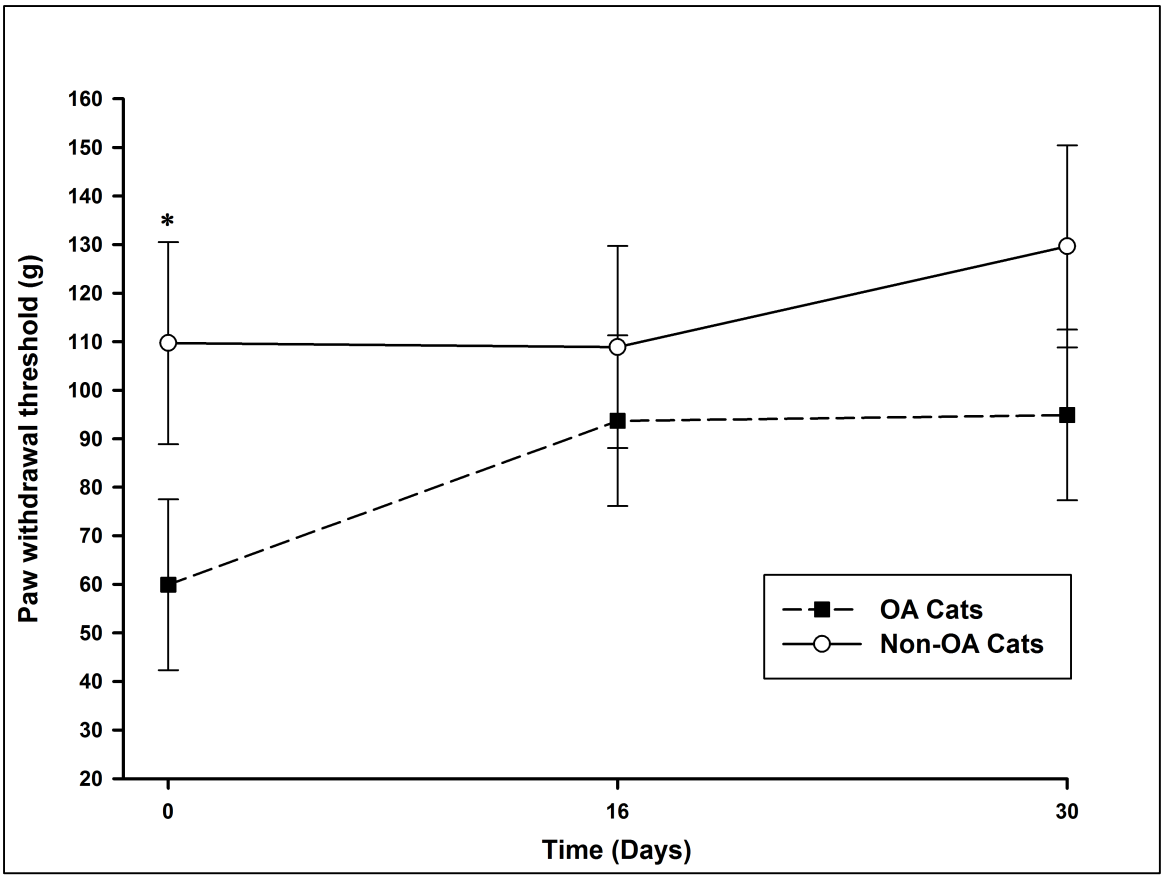
Legend : * $P < 0.10$.

Table 2.4.1b: Phase I Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 3 (MI-CAT(V)-v3) scores, expressed as median (range), over time in osteoarthritic cats (n = 7).

Scale component assessed	Baseline	Day 29	*P*-value
1. Body posture – back	0.5 (0-3)	0 (0-2)	0.090*
2. Body posture – forelimbs	1 (0-3)	1 (0-2)	0.3537
3. Body posture – hind limbs	2.5 (0-8)	2.5 (0-7)	0.200
4. Gait – general	1 (0-5)	1 (0-3)	0.340
5. Gait – forelimbs	1 (0-5)	0 (0-3)	0.055*
6. Gait - hind limbs	1 (0-4)	1 (1-5)	0.090*
7. Willingness and ease of horizontal movements	2.5 (0-8)	2.5 (0-8)	0.4331
8. Standing up on hind feet to investigate a higher surface	0 (0-4)	0 (0-2)	0.0785*
9. Jumping	5 (1-7)	4 (2-6)	0.399
10. Other behaviors	0 (0-2)	0 (0-1)	0.1593
Total MI-CAT(V)-v3 score	14.5 (6-28)	15 (10-25)	0.3687

Legend: Gabapentin was administered three times daily from days 1 to 30. *$P < 0.10$.

Figure 2.4.4: Validation phase I osteoarthritic (OA) *vs.* non-OA group mean (confidence intervals (CIs)) values for paw withdrawal threshold (PWT) over time.



Legend: All cats received gabapentin three times daily from day 1 to 30. Error bars represent 95% CIs. The univariate effect of day was significant in OA ($P < 0.001$) but not in non-OA ($P = 0.075$) cats. * Statistically significant ($P < 0.05$) difference between groups.

Baseline NAM was higher for non-OA than OA cats ($P = 0.01$). Osteoarthritic cats' NAM increased from baseline during gabapentin treatment ($P < 0.0001$), beginning at W1 ($P < 0.0001$) (Table 2.4.1c). Non-OA cats' NAM also increased during treatment ($P < 0.0001$), and remained higher than OA cats' NAM ($P = 0.027$).

Table 2.4.1c: Night-time locomotor activity monitoring (NAM) for osteoarthritic (OA) and non-OA cats over time, expressed as mean (standard error) of the log-plus-one-transformed mean nightly (6 pm to 5:58 am) activity for the period (n = 108 averaged recordings over each period for each cat).

Group	Baseline	Week 1	Week 2	Week 3	Week 4
Non-OA (n = 5)	0.519 (0.037)[a]	0.584 (0.387)[b]	0.615 (0.044)[b]	0.600 (0.022)[b]	0.559 (0.042)
OA (n = 7)	0.350 (0.054)[a]	0.455 (0.035)[c]	0.465 (0.054)[c]	0.521 (0.072)[c]	0.453 (0.063)

Legend: All cats received gabapentin three times daily from weeks 1 to 4. [a] Significant between group difference (P = 0.01). [b, c] Significantly different from baseline ($P < 0.05$).

2.4.4.2 Phase II

Mean age was 3.25 years (range 2.75-4 years) for non-OA and 10.64 years (range 9.75-11.75 years) for OA cats. One OA cat was withdrawn for allergic dermatitis, as described elsewhere.(Monteiro, Klinck et al. 2017) Table 2.4.2a shows baseline MI-CAT(V) results for OA *vs.* non-OA cats; BP-H (P = 0.0074) and jumping (P = 0.085) detected OA status. Total scale inter-rater (≥ 0.85), intra-rater (≥ 0.88) and internal consistency (≥ 0.84) reliability were excellent (Tables 2.4.2b, 2.4.2c, and 2.4.2d).

Table 2.4.2a: Phase II baseline comparison of Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 4 (MI-CAT(V)-v4) scores (based on percentage of maximum possible score; range 0-1), expressed as mean (standard deviation (SD)), for osteoarthritic (OA) and non-OA cats.

Scale component assessed	OA cats n = 15	Non-OA cats n = 5	*P*-value
1. Body posture – back	0.23 (0.15)	0.18 (0.11)	0.5853
2. Body posture – forelimbs	0.08 (0.05)	0.08 (0.03)	0.8785
3. Body posture – hind limbs	0.15 (0.09)	0.04 (0.04)	0.0074*
4. Gait	0.26 (0.19)	0.12 (0.06)	0.1705
5. Willingness and ease of horizontal movements	0.34 (0.15)	0.43 (0.08)	0.1406
6. Jumping	0.25 (0.14)	0.14 (0.06)	0.0895*
7. Other behaviors	0.08 (0.17)	0.00 (0.00)	0.5395
8. Global distance examination (lameness)	0.35 (0.28)	0.12 (0.13)	0.1471
MI-CAT(V)-v4 final score	0.22 (0.09)	0.15 (0.05)	0.1035

Legend: *$P < 0.10$.

Table 2.4.2b: Phase II Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 4 (MI-CAT(V)-v4) inter-rater reliabilities.

Scale component assessed	Day -7 n = 20		Day 52 n = 14	
	ICC	95% CI	ICC	95% CI
1. Body posture – back	**0.833**	**0.578-0.934**	**0.864**	**0.576-0.956**
2. Body posture – forelimbs	0.458	-0.370-0.785	0.464	-0.669-0.828
3. Body posture – hind limbs	0.686	0.207-0.876	**0.833**	**0.479-0.946**
4. Gait	0.676	0.182-0.872	0.687	0.026-0.900
5. Willingness and ease of horizontal movements	**0.778**	**0.438-0.912**	**0.964**	**0.888-0.988**
6. Jumping	**0.883**	**0.704-0.954**	**0.930**	**0.781-0.977**
7. Other behaviors	0.636	0.079-0.856	**0.952**	**0.851-0.985**
8. Global distance examination (lameness)	**0.767**	**0.411-0.908**	**0.775**	**0.261-0.931**
MI-CAT(V)-v4 final score	**0.850**	**0.620-0.940**	**0.896**	**0.675-0.967**

Legend: Bold indicates excellent reliability coefficients. ICC = intraclass correlation coefficient; CI = confidence interval.

Table 2.4.2c: Phase II Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 4 (MI-CAT(V)-v4) intra-rater reliabilities.

Scale component assessed	Days -7 and -3 n = 20		Days 52 and 54 n = 14	
	ICC	95% CI	ICC	95% CI
1. Body posture – back	**0.770**	**0.420-0.909**	**0.745**	**0.204-0.918**
2. Body posture – forelimbs	0.433	-0.432-0.776	**0.850**	**0.532-0.952**
3. Body posture – hind limbs	**0.797**	**0.487-0.920**	**0.794**	**0.360-0.934**
4. Gait	**0.917**	**0.791-0.967**	**0.918**	**0.744-0.974**
5. Willingness and ease of horizontal movements	0.466	-0.349-0.789	**0.857**	**0.556-0.954**
6. Jumping	**0.837**	**0.588-0.935**	0.546	-0.415-0.854
7. Other behaviors	0.689	0.215-0.877	**0.864**	**0.575-0.956**
8. Global distance examination (lameness)	**0.962**	**0.903-0.985**	**0.978**	**0.927-0.993**
MI-CAT(V)-v4 final score	**0.885**	**0.709-0.954**	**0.897**	**0.679-0.967**

Legend: Bold indicates excellent reliability coefficients. ICC = intraclass correlation coefficient; CI = confidence interval.

Table 2.4.2d: Phase II Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 4 internal consistency reliabilities based on experienced evaluator's assessments, expressed as Cronbach's alpha (95% confidence interval).

Assessment time	OA cats n = 15	Non-OA cats n = 5	All cats n = 20
Day -7	0.847 (0.69-0.95)	0.743 (0.25-0.97)	0.842 (0.71-0.93)
Day -3	0.858 (0.72-0.95)	0.810 (0.35-0.99)	0.880 (0.78-0.95)

Neither tramadol treatment, week nor their interaction significantly affected total MI-CAT(V) score ($P > 0.26$), despite responses of objective outcomes (PVF, NAM and RMTS), as described elsewhere.(Monteiro, Klinck et al. 2017) Gait ($P = 0.0199$) and GDE ($P =$

0.0682) decreased with week (period 1, tramadol group). Gait improved with OTMS (P = 0.096), and BP-H with tramadol + OTMS (P = 0.042), but total MI-CAT(V)-v4 score did not (P > 0.79) (period 2); objective outcomes responded to treatment (PVF in both groups, NAM in OTMS, and RMTS in tramadol + OTMS), as described elsewhere.(Monteiro, Klinck et al. 2016)

2.4.4.3 Phase III

Mean age was 3.38 years (range 2.75-4 years) for non-OA and 10.78 years (range 9.75-11.75 years) for OA cats. Scale completion took approximately 10 minutes per cat. Total MI-CAT(V)-v5 score, and all subcategories except WEHM, detected OA status (Table 2.4.3a). Total score reliability was good to excellent for inter-rater (0.64-0.75) and excellent for intra-rater (0.79-0.91) reliability; internal consistency was acceptable (overall α = 0.85) (Tables 2.4.3b, 2.4.3c and 2.4.3d). Only jumping improved (P = 0.064) with tramadol treatment; total MI-CAT(V)-v5 score did not (P = 0.9347), nor did treatment and placebo effects on scores differ (P = 0.4244), despite positive responses of objective outcomes (PVF, NAM and RMTS), such as described elsewhere.(Monteiro, Klinck et al. 2017) Worsening of WEHM (P =0.028) occurred with tramadol treatment.

Table 2.4.3a: Phase III baseline comparison of Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 5 (MI-CAT(V)-v5) scores (based on percentage of maximum possible score; range 0-1), expressed as mean (SD) for osteoarthritic (OA) and non-OA cats.

Scale component assessed	OA cats n = 13	Non-OA cats n = 6	*P*-value
1. Body posture	0.30 (0.10)	0.11 (0.03)	0.0006*
2. Gait	0.28 (0.20)	0.04 (0.05)	0.0031*
3. Willingness and ease of horizontal movements	0.24 (0.15)	0.15 (0.05)	0.2020
4. Jumping	0.30 (0.19)	0.13 (0.14)	0.0824*
5. Global distance examination (lameness)	0.37 (0.23)	0.05 (0.05)	0.0001*
Total MI-CAT(V)-v5 score	0.30 (0.12)	0.09 (0.04)	0.0001*

Legend: * $P < 0.10$.

Table 2.4.3b: Phase III Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 5 (MI-CAT(V)-v5) inter-rater reliabilities.

Scale component assessed	Day -13 n = 19		Day 17 n = 13	
	ICC	95% CI	ICC	95% CI
1. Body posture	0.70	0.37-0.87	0.24	0.00-0.69
2. Gait	0.59	0.20-0.82	0.47	0.00-0.80
3. Willingness and ease of horizontal movements	0.61	0.23-0.83	0.70	0.26-0.90
4. Jumping	0.50	0.07-0.77	0.40	0.00-0.77
5. Global distance examination (lameness)	0.72	0.41-0.88	0.66	0.20-0.88
Total MI-CAT(V)-v5 score	**0.75**	**0.46-0.90**	0.64	0.17-0.88

Legend: Bold indicates excellent reliability coefficients. ICC = intraclass correlation coefficient; CI = confidence interval.

Table 2.4.3c: Phase III Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 5 (MI-CAT(V)-v5) intra-rater reliabilities.

Scale component assessed	Days -13 and -1 n = 19		Days 17 and 19 n = 13	
	ICC	95% CI	ICC	95% CI
1. Body posture	**0.79**	**0.54-0.91**	0.66	0.20-0.88
2. Gait	**0.85**	**0.65-0.94**	**0.86**	**0.60-0.95**
3. Willingness and ease of horizontal movements	0.45	0.01-0.74	0.31	0.00-0.72
4. Jumping	0.50	0.08-0.77	0.66	0.18-0.89
5. Global distance examination (lameness)	**0.97**	**0.92-0.99**	**0.92**	**0.77-0.98**
Total MI-CAT(V)-v5 score	**0.91**	**0.79-0.97**	**0.90**	**0.72-0.97**

Legend: Bold indicates excellent reliability coefficients. ICC = intraclass correlation coefficient; CI = confidence interval.

Table 2.4.3d: Phase III Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians version 5 internal consistency reliabilities based on experienced evaluator's assessments, expressed as Cronbach's alpha (95% confidence interval).

Assessment time	OA cats (n = 13)	Non-OA cats (n = 6)	All cats (n = 19)
Day -1	0.73 (0.41-0.92)	0.12 (-1.67-0.86)	0.85 (0.72-0.94)

2.4.5 Discussion

The MI-CAT(V) was designed to complement veterinary examination of cats at risk for OA, but it previously lacked sensitivity.(Klinck, Rialland et al. 2015) This report describes scale refinement and validation. Phases I-III assessed various versions of the revised scale for ability to detect OA status and treatment effects, with modifications (*e.g.*, item removal, wording/scoring changes), based on item/subcategory performance. The resulting MI-CAT(V)-v5 distinguished OA from non-OA cats and was reliable. It did not detect OA treatment effects, despite apparent treatment responsiveness of some subcategories in each phase, and despite responses of objective measures (PVF, NAM, RMTS; reported elsewhere) to treatments.(Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017)

In phase I, it was expected that several new scale items would contribute "noise" to the total score or might be miscoded; indeed, only one MI-CAT(V)-v3 subcategory, WEHM, detected OA. The SOE did not discriminate OA status, aligning with previous findings.(Klinck, Rialland et al. 2015, Lascelles, Dong et al. 2012) Detection of a gabapentin treatment effect in OA but not in non-OA cats by three scale subcategories, BP-B, G-F, and SUHF, and SOE distance score was cautiously interpreted (owing to the lack of a placebo group for comparison, and results of objective assessments described above and discussed below) as promising. The distance score subsequently included in the MI-CAT(V)-v4 is comparable to lameness scores used in other species (*e.g.*, dog, horse, cow, sow) but not reported in cats.(Ashley, Waterman-Pearson et al. 2005, Flower and Weary 2006, Ison,

Clutton et al. 2016, Quinn, Keuler et al. 2007) Prior reports conflict regarding lameness as a feature of feline OA.(Bennett, Zainal Ariffin et al. 2012, Klinck, Frank et al. 2012, Lascelles 2010)

In phases II and III, OA detection improved, with the MI-CAT(V)-v5 total score and most scale subcategories detecting OA. This was despite a reduction in the number of items, and it supports the scale refinement made between study phases, particularly with respect to the selection of items for retention *vs.* removal. Disappointingly, total MI-CAT(V) score detected no treatment effects in the placebo-controlled trials of phases II and III, though objective measures,(Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017) and individual subcategories (BP-H, gait, jumping), did. Difficulty detecting therapeutic responses with the MI-CAT(V) and SOE underscores the challenges facing veterinarians when evaluating OA pain in cats.

Scale internal consistency between 0.70 and 0.90 (for OA cats, or all cats) indicated item relatedness, without redundancy.(Streiner and Norman 2008) Lower internal consistency in non-OA cats (phase III) is of little concern (the scale targets cats with/at risk for OA), and was likely due to fewer scale items and sample homogeneity.(Bartlett and Frost 2008, Streiner and Norman 2008) However, it should be noted that the large number of scale items likely inflated the Cronbach's α results; future, larger-scale studies should assess relationships between scale components *via* factor analysis. Total score inter- and intra-rater reliability were good to excellent. Inter-rater and intra-rater reliability for most scale subcategories was good to excellent in phase II, with mild inter-rater reliability improvements between assessments. Detailed evaluation instructions and training prior to scale use minimized the impact of inexperience with the scale (naïve evaluator). Weaker subcategory inter- and intra-rater reliabilities in phase III may have resulted from the reduced number of scale items. The GDE subcategory performed generally well. Gait had lower inter- than intra-rater reliability, suggesting a systematic difference between evaluators; more user training may be needed. The same was the case for jumping in phase III. Subcategories BP-B and BP-H performed well, but BP-F performed similarly to gait (phase II), and body posture (phase III) had inconsistent inter-rater reliability, again suggesting a need for more user training. Better WEHM inter- than

intra-rater reliability could indicate day to day instability, which, combined with poorer OA detection and therapeutic responsiveness in phase III, may warrant item revisions or removal. However, tramadol may have behavioral effects (*e.g.*, sedation, agitation, dysphoria); such effects could have contributed to WEHM's poor response to treatment.(Beakley, Kaye et al. 2015, Wright and Rychel 2013) Elimination of several jumping and WEHM items after phase II may have decreased reliability.

Gabapentin has been recommended for feline neuropathic and OA pain.(Mathews 2008, Rychel 2010) To our knowledge, this is the first reported therapeutic trial of gabapentin in feline OA,(KuKanich 2013) although one case report described a positive response.(Lorenz, Comerford et al. 2013) Improvements in PWT and NAM are promising, despite the small number of cats and lack of a placebo group; however, OA cats without central sensitization may respond differently to gabapentin. Therapeutic responsiveness of AM in feline OA is well established for meloxicam (oral suspension or OTMS), tramadol, a therapeutic diet and anti-NGF monoclonal antibody,(Gruen, Thomson et al. 2016, Guillot, Moreau et al. 2013, Lascelles, Hansen et al. 2007, Lascelles, DePuy et al. 2010, Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017) but high inter-individual variability generally limits comparisons between individuals.(Andrews, Potter et al. 2015) Baseline NAM in phase I distinguished non-OA from OA cats. Greater age in OA than in non-OA cats could have contributed to this, but previous studies having similar sample sizes and greater OA *vs.* non-OA group age disparities have not reported distinction of OA status.(Guillot, Chartrand et al. 2015, Guillot, Moreau et al. 2012, Guillot, Moreau et al. 2013, Monteiro, Klinck et al. 2017) All participating OA cats had evidence of central sensitization (allodynia), which affects a subset of humans and cats with OA;(Guillot, Moreau et al. 2013, Lluch, Torres et al. 2014) this may have contributed to activity differences between OA and non-OA cats. Osteoarthritic cats had decreased allodynia on gabapentin (beginning D16); non-OA cats' PWT also tended to increase, later in treatment. Based on the latter and the small sample size, further research is needed to confirm effects of gabapentin on central sensitization, as measured by PWT. Osteoarthritic and non-OA cats' NAM both increased (beginning W1). Undetected causes of neuropathic pain, other than OA, could have influenced non-OA cat activity, and responded to gabapentin. Alternatively, gabapentin or other study influences may have had non-analgesic effects on both groups'

NAM. Different aspects of OA pain are measured by PWT and NAM; PWT appears less susceptible to non-specific effects of gabapentin. The combination of ability to detect feline OA-associated central sensitization,(Guillot, Moreau et al. 2013) its apparent response to treatment with gabapentin and tramadol, (Monteiro, Klinck et al. 2017) and the recent finding that it is moderately reliable, valid and clinically feasible in naïve, client-owned cats,(Addison and Clements 2017) suggest further investigation of PWT as a diagnostic modality for feline clinical practice would be worthwhile.

There is a lack of consensus on the determination of sample sizes in scale validation studies, but our samples were small compared to those typically recommended for human health measurement scale validation.(Anthoine, Moret et al. 2014) This, and the use of the same cats in phases II and III, could have favored selection of OA characteristics particular to the sample. Results also may not translate from the laboratory colony to the clinical setting (*e.g.*, owing to poorer compliance of client-owned cats, variability in the time cats have to acclimate to the examination room and examiner, or the influences of unrelated procedures on cat behavior). We would argue that many cats can be persuaded, using treats, vocal encouragement, petting, brushing, *etc.*, to move about an examination room,(Kerwin 2012) giving the MI-CAT(V) potential for clinical application.

Feline OA evaluation relies heavily on owner report;(Klinck, Frank et al. 2012) some owner pain scales distinguish OA from non-OA cats and detect treatment effects.(Benito, Depuy et al. 2013, Bennett and Morton 2009, Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Lascelles, Hansen et al. 2007) However, in the absence of accurate owner report (*e.g.*, research or homeless cats, or inattentive or medically/cognitively impaired owners), a valid OA scale for veterinarians could be particularly useful. Future, larger-scale studies, with different classes of analgesics, are needed to confirm MI-CAT(V) reliability and ability to detect OA, particularly in client-owned cats in a clinical setting, and to determine its feasibility. Thresholds must also be established for determination of OA status. Finally, responsiveness to treatment requires improvement if the MI-CAT(V) is to be used for more than OA screening.

2.4.6 Conclusions

The MI-CAT(V) was reliable and distinguished OA from non-OA cats, giving it potential for screening of at-risk cats. Owing to study limitations, further assessment is needed to confirm this potential. Although individual subcategories showed promise, total MI-CAT(V) score did not detect treatment effects, limiting its current utility in veterinary case management.

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2.4.8 Conflicts of interest

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3. DISCUSSION

3.1 Overview

The development and validation of standardized pain rating scales is a complex process. At the time that this project began, the CSOM was the only scale that had been evaluated for use in feline OA (Lascelles, Hansen et al. 2007); no standardized multi-item scales had been reported. The studies described in the preceding section detail the development of two such scales, based on review of the literature, expert opinion in feline pain and behavior, and owner survey and review of veterinary diagnostic methods in 50 cases of feline OA (Klinck, Frank et al. 2012). The MI-CAT(C), for use by cat owners, and the MI-CAT(V), for use by veterinarians, then underwent a content validation phase *via* expert review, and a preliminary evaluation of validity (ability to detect OA) and reliability (inter-rater, intra-rater, and internal consistency) *via* a pilot study in a group of laboratory cats (Klinck, Gruen et al. 2017, Klinck, Rialland et al. 2015).

The MI-CAT(C) did not detect OA status in the pilot study; reliability of items was variable. Based on cautious interpretation (due to the differences of the study sample from target population, as discussed below) of the findings, some poorly performing items were removed. At this point, the MI-CAT(C) was determined not to be further evaluable in laboratory cats. This was because of substantial differences in the laboratory environment *vs.* the home environment (*e.g.*, decreased complexity and lack of some aspects such as stairs), and in the relationships with the persons conducting evaluations, *i.e.*, animal care attendants and veterinary technicians *vs.* owners. The MI-CAT(C) was to be completed based on general recollection of the cat's behavior; animal caregivers in the laboratory simply did not spend as much time with cats as do most owners (*e.g.*, being absent overnight), nor did they have the same types of interactions with the cats (*e.g.*, most contact involved active care such as feeding and cleaning, but not inactive time such as simply sitting with or in the same room as the cat for extended periods). The scale was therefore tested in a placebo-controlled, double-blinded trial of meloxicam, in client-owned cats with clinical (joint pain and mobility impairment) and radiographic evidence of DJD (Klinck, Gruen et al. 2017). This evaluation

provided support for the scale's reliability (intra-rater and internal consistency; evidence of validity based on internal structure) and treatment responsiveness (evidence of validity based on response processes; construct validity) in the population sampled, as well as its comprehension by cat owners (face validity) (Klinck, Gruen et al. 2017).

In the pilot study, the MI-CAT(V)'s reliability was acceptable, but the scale was unable to detect OA status (Klinck, Rialland et al. 2015). In contrast to the MI-CAT(C), the MI-CAT(V) was considered to be further evaluable in laboratory cats with and without naturally-occurring OA, because it was dependent on an evaluation procedure conducted in a brief window of time and in an examination room context. Indeed, the laboratory context permitted evaluation of this scale under standardized conditions, thereby minimizing the confounding influences of extraneous sources of error associated with a clinical setting. Consequently, this scale underwent a series of refinements and revisions in a laboratory setting, following development and preliminary validity assessments in the pilot study (Klinck, Monteiro et al. 2017). Initially, a video analysis of OA and non-OA cats was conducted to identify new items to expand the scale, with the goal of increasing sensitivity to OA. Substantial revisions were performed on the basis of the results. Next, the MI-CAT(V) was assessed in three consecutive therapeutic trials, and revisions were made to the scale at each step, based on scale subcategory and individual item performance (particularly with respect to reliability and capacity to distinguish OA from non-OA cats). First, a gabapentin trial was conducted in OA and non-OA cats, which supported scale reliability and the ability of some scale subcategories and items to detect OA status and response to treatment, and allowed for further revisions. Of particular note was the inclusion of a new item from the surgeon's evaluation, the Global Distance Examination. Second, a placebo-controlled, double-blinded study was conducted, involving an initial tramadol or placebo treatment period followed by an oral transmucosal meloxicam spray, or oral transmucosal meloxicam spray with tramadol, treatment period. Third, OA and non-OA cats were again evaluated with the scale in the context of a double-blinded, placebo-controlled tramadol study. In each of the last two phases, comparisons between non-OA and OA cats, OA cats before and after treatment, and within and between veterinarian scale users, were performed. Acceptable inter- and intra-rater, and internal consistency reliability (evidence of validity based on internal structure) was

maintained through the second and third therapeutic trial studies, and the final version of the MI-CAT(V) distinguished OA from non-OA cats (evidence of validity based on response processes; construct validity), but did not respond to treatment.

The process described above resulted, for both scales, in partial validation, with each one showing promise, but neither ready for use as a clinical decision-making tool at this time. Contributions of this work to the field of feline medicine, limitations of the research project, and recommendations for further study, are discussed below.

3.2 Contributions to the field of feline medicine

Other owner pain scales have been reported for feline OA, with one, the FMPI, distinguishing OA from non-OA cats (Benito, Depuy et al. 2013). The FMPI (Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Gruen, Thomson et al. 2016) and two others, the CSOM (Gruen, Thomson et al. 2016, Lascelles, Hansen et al. 2007) and a standardized scale reported by Bennett and Morton (Bennett and Morton 2009) have also been found to detect treatment effects. However, difficulties remain, particularly in distinguishing treatment from placebo effect; there was no placebo group in the reported testing of the Bennett and Morton scale, and the other two scales have had variable success in distinguishing treatment from placebo effects, from trial to trial (Benito, Hansen et al. 2013, Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015, Gruen, Thomson et al. 2016, Lascelles, DePuy et al. 2010). Owner pain scales have not been specifically evaluated for their ability to distinguish feline OA pain and disability from that related to other disease. In addition, although feline orthopedic examination findings such as pain, palpable abnormalities, and goniometry have been evaluated for concordance with radiographic signs of OA or DJD (Clarke and Bennett 2006, Lascelles, Dong et al. 2012, Lascelles, Hansen et al. 2007), there have been no other reports of veterinarian pain scales for feline OA. The MI-CAT(V) is therefore the first reported standardized pain scale developed for assisting the veterinarian in detecting OA pain in cats, during the physical examination. This is of value because there may be cases in which owner report is unavailable (*e.g.*, absent, inattentive, cognitively impaired or ill owners, or cats that do not live in a typical home setting, such as research or breeding colony cats, or

homeless/rescued cats). The veterinary tool also may assess different aspects of feline OA than do the owner pain scales (*e.g.*, sensory *vs.* emotional dimension). With respect to the MI-CAT(C), although other owner scales (FMPI, CSOM, and Bennett and Morton scale) exist, none is perfect and there remains no gold standard for assessing feline OA pain. The MI-CAT(C) differs substantially from the CSOM (a personalized scale based on the particular cat's activities affected by DJD, that can be used to compare a single patient over time) (Lascelles, Hansen et al. 2007) and the Bennett and Morton scale (which asks owners to rate mobility, activity, grooming habits, temperament, and overall severity, each on a scale of 1-10, and provides examples of behaviors to consider when making the assessment) (Bennett and Morton 2009). It is most similar to the FMPI (Benito, Depuy et al. 2013, Benito, Hansen et al. 2013, Gruen, Griffith et al. 2014, Gruen, Griffith et al. 2015), in that it asks the owner to evaluate specific, defined activities; however, the MI-CAT(C) uses more items and provides simplified response options, as compared to the FMPI, which may result in differences in performance between these two scales. In other species such as the dog, multiple OA pain scales also exist (*e.g.*, the Canine Brief Pain Inventory (Brown, Bell et al. 2013, Brown, Boston et al. 2008, Brown, Boston et al. 2007), Helsinki Chronic Pain Index (Hielm-Björkman, Rita et al. 2009), Liverpool Osteoarthritis in Dogs index (Hercock, Pinchbeck et al. 2009, Walton, Cowderoy et al. 2013), and CSOM (Muller, Gaines et al. 2016, Rialland, Bichot et al. 2012)). The MI-CAT(C) developed as a part of this project contributes to the body of understanding regarding OA in cats, and adds to the tools available for assessing it, without replacing other owner pain scales.

3.2.1 Owner survey contributions to the understanding of feline osteoarthritis

The survey of owners of cats with OA provided both new information and support for previous findings regarding feline OA (Klinck, Frank et al. 2012). First, veterinarians in the sample relied heavily on owner report to make their diagnosis of OA, only very rarely detecting the disease in the absence of owner observations consistent with OA (*i.e.*, based on their physical examination findings alone). Second, even signs reportedly seen in the home were not necessarily detected by veterinarians during the clinical examination (*e.g.*, gait

abnormalities), and abnormalities or pain upon palpation and manipulation of the joints were relatively uncommon. The reasons for this are unclear; cats may mask signs of disease in a threatening environment, such as in the veterinary clinic, and/or veterinarians may not perform a comprehensive orthopedic examination, either due to poor cat compliance (*e.g.*, with palpation and manipulation) or due to perceived practical difficulties (*e.g.*, in conducting an evaluation of gait at a distance) (Kerwin 2012). This disconnect between physical/orthopedic examination findings and historical findings underscores deficiencies of the veterinary orthopedic examination in cats, reinforcing the potential usefulness of the MI-CAT(V). Conversely, it confirms the importance of careful questioning of owners of at-risk cats, regarding the presence of signs of OA in the home. Previous reports have described the disconnect between radiographic signs of OA and orthopedic examination abnormalities (Clarke and Bennett 2006, Lascelles, Dong et al. 2012, Lascelles, Hansen et al. 2007). The difficulty of confirming the presence of joint pain upon palpation and manipulation, in cats, has been discussed (Bennett, Zainal Ariffin et al. 2012). Interestingly, in the owner survey described here, the numbers of cats diagnosed with OA were quite low at each participating clinic, supporting the notion that feline OA remains difficult to diagnose. While careful owner questioning has been recommended to improve detection of OA in at-risk cats (Bennett, Zainal Ariffin et al. 2012, Lascelles and Robertson 2010), it could be hypothesized that reliance primarily on owner reports to detect OA in cats may still result in under-diagnosis (*e.g.*, if owners are not attentive or otherwise unable to detect and report OA signs).

The most common signs of OA reported by owners in the survey were changes in mobility (*e.g.*, gait, jumping, stair use). Changes were also described in activity and time spent resting, self-grooming, social behavior with family members and other pets, mood, litter box use, play and hunting behaviors, posture, selection of resting areas, and vocalization and objection to handling. This was generally consistent with what had been previously described in the literature; however, gait changes were the most common abnormality. The prevalence of lameness/stiffness in feline OA has been reported to be low; authors have questioned the importance of gait changes in the disease in this species, noting that they could be less prominent due to the cat's small size and the prevalence of bilateral disease (Bennett, Zainal Ariffin et al. 2012, Clarke and Bennett 2006, Guillot, Gravel et al. 2015, Hardie, Roe et al.

2002, Slingerland, Hazewinkel et al. 2011). The findings of this owner survey study support their importance in at least a subset of OA cats.

A high prevalence of other geriatric diseases was found in the owner survey sample of cats diagnosed with OA. Other research has found a higher than expected concurrence of DJD with CKD, in cats (Marino, Lascelles et al. 2014). The authors of the latter study suggested as one possible explanation that inflammatory or immune-mediated etiologies may have contributed to the development of both CKD and DJD. This could conceivably also be the case for other diseases found to be prevalent in the owner survey (*e.g.*, cardiac disease, diabetes mellitus), but would require confirmation. See further discussion below.

Veterinarians in the sample most often prescribed DMOADs such as glucosamine, pentosan PS, or PSGAGs for treatment of OA. Such treatments are expected to be disease-modulating and may or may not be analgesic (Kerwin 2010, McNamara, Johnston et al. 1997). There exists limited evidence to support that they alleviate signs of clinical OA in cats (Sul, Chase et al. 2014). One feline study did find that a therapeutic diet rich in omega-3 fatty acids, and supplemented with glucosamine and chondroitin sulfate, and green-lipped mussel extract, improved activity and some behavioral indices of DJD pain in cats, when compared to a control diet (Lascelles, DePuy et al. 2010). Therapeutic options are limited in cats (only meloxicam is approved for long-term use in chronic musculoskeletal disease, and that only in limited markets, not including Canada and the USA), and veterinarians may avoid prescription of NSAIDs and other analgesics for long-term use, due to concern over potential for adverse effects (Sparkes, Heiene et al. 2010). It is possible that DMOADs were prescribed preferentially in this sample due to perceived lower risk than for other therapies, such as NSAIDs.

3.2.2 Contributions to the detection, measurement, and management of feline osteoarthritis

3.2.2.1 The Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner

The MI-CAT(C) is a pain scale that was intended to be completed by caretakers/owners of cats at risk for OA, to aid in the detection of pain associated with the disease. The results of this research provide support for its reliability and validity when used to assess therapeutic response in client-owned OA cats (Klinck, Gruen et al. 2017). At this point, the scale may be used for subjective assessments of therapeutic efficacy, in a research context, as it has shown an ability to distinguish treatment from placebo. However, it is not yet ready for use as a clinical decision-making tool in individual cats.

Expert review of the MI-CAT(C) and comparison with owner survey results supported the comprehensiveness, representativeness, and clarity of the scale (evidence of validity based on content). The scale differentiated placebo from meloxicam treatment in OA cats (evidence of validity based on response processes; construct validity) and demonstrated convergence with: 1) objective activity monitoring, 2) another subjective measure, the CSOM, and 3) age, which is the single most important predictor of the presence of OA (Lascelles, Henry III et al. 2010, Slingerland, Hazewinkel et al. 2011) (evidence of validity based on relations to other variables; construct validity). Owners found most scale items clear and easy to understand, generally supporting both comprehensibility and acceptability (face validity) of the scale.

Internal consistency reliability for the MI-CAT(C) total fell into an acceptable range between 0.70 and 0.90, supporting that scale components are sufficiently related to each other, without being redundant (Streiner and Norman 2008); however, as discussed below, this result must be interpreted somewhat cautiously due to the large number of scale items. Intra-rater reliability was generally acceptable for the MI-CAT(C) total and subcategory scores; it was lowest for the Physical Condition (abnormalities) subscale. All items of the latter subcategory demonstrated some trouble with comprehension (several owners reported difficulty understanding items); poor comprehensibility could have contributed to lower reliability. Inter-rater reliability of the MI-CAT(C) was only fair to good, and poorer than intra-rater

reliability. This, combined with widely variable degrees of concordance between different pairs of owners, suggests that the MI-CAT(C) may be substantially influenced by owner familiarity with the cat and therefore that it should be completed by the primary owner. Inter-rater reliability is generally expected to be lower than intra-observer reliability (Streiner and Norman 2008), and it has been noted that the former is particularly important for observer pain scales (Gélinas, Loiselle et al. 2008); however, in the case of client-owned cats, it is logical that certain household members would be more involved in cat care, and more familiar with the cat, than others. Investigations of differences between owners with respect to reporting of OA signs, either generally, or with respect to other feline pain scales, have not been described. However, the potential for differences in owner familiarity with pet cats may warrant consideration when questioning of owners of at-risk cats for signs of OA.

3.2.2.2 The Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians

The MI-CAT(V) is a pain scale designed for completion by veterinarians, to augment the physical examination of cats at risk for OA. The results of this research provide preliminary support for its reliability and ability to distinguish OA from non-OA cats; however, further evaluation and refinement are needed. The MI-CAT(V) has thus far not been found to be able to detect treatment effects; therefore, it has potential for future use in screening for feline OA, either alone, or in combination with an owner scale such as the FMPI (*e.g.*, to increase diagnostic certainty). However, because testing has only been performed in laboratory cats and cut-points for OA detection have not been established, it is not ready for such use at this time. See further discussion, below.

Preliminary content for the scale was based on a review of the literature (Bennett and Morton 2009, Clarke and Bennett 2006, Godfrey 2005, Gunew, Menrath et al. 2008, Hardie, Roe et al. 2002, Lascelles 2010, Lascelles, Hansen et al. 2007, Lascelles and Robertson 2010, Lascelles, DePuy et al. 2010, Lascelles, Henry III et al. 2010, Scarlett and Donoghue 1998, Zamprogno, Hansen et al. 2010) and clinical expertise. Expert review of the MI-CAT(V) supported its comprehensiveness and the appropriateness of response options, as well as its clarity (evidence of validity based on content). Review by veterinary students supported comprehensibility and acceptability (face validity), although individual comments regarding

the potential for temperament to influence Exploratory (subsequently part of Willingness and Ease of Horizontal Movements, and Jumping) and Interactive Behavior (later eliminated), and for Gait not to be evaluable in the clinic, indicated aspects of the scale that might be less well accepted.

The series of experiments conducted in laboratory cats with and without naturally-occurring OA permitted substantial revisions to this scale. In its first iteration following content validation (MI-CAT(V)-v1), it contained items in the subcategories of Exploratory Behavior, Body Posture, Gait/Locomotion (Gait), Interaction with Examiner, Body Condition Score, Coat Condition, Claw Condition, Palpation and Manipulation – Findings, and Palpation and Manipulation – Cat Response. Gait, Body Posture, and Exploratory Behavior showed the most promise with respect to their capacity to detect OA status (evidence of validity based on response processes; construct validity), in the first stages of validation. However, distinction of OA from non-OA cats by these scale components was either not consistently significant, or statistically insignificant (a tendency only), indicating inadequate sensitivity to OA. Gait and some aspects of Body Posture were correlated with an objective measure of OA, PVF (evidence of validity based on relations with other variables; convergent, construct validity). Based on these results, it was considered plausible that Gait and Body Posture could reflect biomechanical effects of OA pain, while Exploratory Behavior and Interaction with Examiner might be influenced more by the affective dimension of OA pain, in addition to cat temperament. Inter-item correlations (internal consistency; evidence of validity based on internal structure) supported retention of Gait, Body Posture, Exploratory Behavior, and Interaction with Examiner, and both inter- and intra-rater reliability were acceptable for all but Gait. Inter-rater reliability increased from the first to second assessment, possibly due to an effect of increasing familiarity with the scale, in the second user. The latter suggested that training would be appropriate prior to scale use.

Items relating to Body Condition Score, Coat, Claws, Palpation and Manipulation - Findings and Palpation and Manipulation – Cat Response were eliminated from the MI-CAT(V) following the pilot study and the main study comparing OA and non-OA cats. This was based on evidence of poor reliability (Body Condition Score, Palpation and Manipulation

– Cat Response) combined with: 1) an absence of sensitivity to OA, *i.e.*, a lack of evidence of validity based on response processes (poor construct validity; Body Condition Score, Palpation and Manipulation – Cat Response, Palpation and Manipulation - Findings, Coat, Claws), and/or 2) a lack of correlation with more promising items (Body Condition Score, Palpation and Manipulation - Findings, Coat, Claws), and/or 3) sign rarity (floor effects; Palpation and Manipulation - Findings). In some cases, subcategories did not have strong support in the literature for their inclusion (Body Condition Score) (Clarke, Mellor et al. 2005, Lascelles, Henry III et al. 2010, Lund, Armstrong et al. 2005, Slingerland, Hazewinkel et al. 2011), or were considered to be easily influenced by owner interventions or by the presence of other diseases (Body Condition Score, Coat, Claws), and therefore either better assessed *via* an owner scale, or not specific to OA.

Veterinary clinical evaluation for OA traditionally relies on joint palpation and manipulation (for signs of pain and for physical abnormalities such as joint thickening or effusion, crepitus, and reduced range of motion); however, physical findings may be difficult to detect in, and also have low sensitivity to, feline OA (Bennett, Zainal Ariffin et al. 2012, Clarke and Bennett 2006, Lascelles, Dong et al. 2012, Slingerland, Hazewinkel et al. 2011). Apparently painful reactions to joint palpation and manipulation may be difficult to interpret in cats, and the finding of pain does not necessarily correlate closely with the presence of radiographic OA (Bennett, Zainal Ariffin et al. 2012, Clarke and Bennett 2006, Lascelles, Hansen et al. 2007). However, due to its importance in clinical practice, Palpation and Manipulation – Cat Response was evaluated with different types of response options involving different degrees of evaluator interpretation (*i.e.*, either simply checking off behavioral responses displayed by the cat, as for the MI-CAT(V)-v1, *vs.* scoring the presence/intensity of pain inferred during palpation and manipulation, as for the MI-CAT(V)-v2). Given that neither method was reliable nor tended to detect OA cats (in fact, with the second method of scoring, non-OA cats had higher joint pain scores than did OA cats), this evaluation was eliminated from the scale. Palpation and Manipulation – Cat Response performed poorly in spite of the fact that cat OA status had been established partly on the basis of palpation findings at screening for inclusion (suggesting a lack of reliability). It is possible that other factors, such as cat temperament, may have influenced Palpation and Manipulation – Cat Response.

Based on the scale's inability to detect OA status in either of the first two versions (MI-CAT(V)-v1 and MI-CAT(V)-v2), but the apparent promise shown by some scale components, a video analysis was undertaken, in which blinded evaluators identified observable differences between OA and non-OA cats; with the goals of: 1) generating more and/or better items relating to Gait, Body Posture, and Exploratory Behavior, 2) noting other potential items based on observable differences between OA and non-OA cats; and 3) establishing more detailed instructions for the evaluation procedure. Major revisions to the scale ensued, affecting items and their structure, scoring, and scale instructions, as well as the incorporation of user training. The third iteration of the scale, the MI-CAT(V)-v3, was thus composed of 67 items in the following 10 subcategories: Body Posture – Back, Body Posture – Forelimbs, Body Posture – Hind Limbs, Gait – General, Gait – Forelimbs, Gait – Hind Limbs, Willingness and Ease of Horizontal Movements, Standing Up on Hind Feet to Investigate a Higher Surface, Jumping, and Other Behaviors.

The subsequent therapeutic trials involving OA and non-OA cats investigated the MI-CAT(V)'s reliability and its ability to distinguish OA from non-OA cats, as well as its responsiveness to treatment in OA cats (both constituting evidence of validity based on response processes, or construct validity), and its relationships with objective measures of OA pain (evidence of validity based on relations with other variables; convergent, construct validity). Alpha level was set at 0.10 for analyses involving the scale (and the surgeon's subjective assessment in the first trial), due to the exploratory nature of the research. Removal of items necessarily decreases true variability between subjects (based on reliability theory) (Streiner and Norman 2008). It was therefore considered more important, at this stage in development and validation, to ensure retention of potentially promising items than to eliminate less promising items.

The first therapeutic trial (gabapentin) permitted evaluation of the new version of the scale (MI-CAT(V)-v3) and its subcategories, and individual item analysis. Because the video analysis was performed by observers blinded to cat OA status, it generated items simply based on differences between cats, without directionality. Therefore, some items were expected to be included either that were not related to OA (contributing "noise" to the scale) or that were

miscoded (*i.e.*, formulated so that OA cats would be scored as less impaired than non-OA cats). This trial included repeated assessments of cats by a board-certified veterinary surgeon, enabling the various aspects of the orthopedic examination to be assessed in parallel with the MI-CAT(V)-v3. The finding that neither pain nor other (physical) abnormalities upon joint palpation and manipulation distinguished between OA and non-OA cats, nor detected a treatment response in OA cats, provided further support for the earlier elimination of those items from the MI-CAT(V). Osteoarthritic cats in this study were required to show evidence of allodynia, for inclusion (and non-OA cats were required not to have evidence of allodynia). The presence of increased pain sensitivity could have been expected to result in more painful reactions to manipulation of OA joints, but it did not seem to do so. This underscores the recognized difficulties in interpreting feline responses to orthopedic manipulations (Bennett, Zainal Ariffin et al. 2012, Kerwin 2012). Conversely, the surgeon's distance (lameness) examination appeared to detect a treatment effect of gabapentin in OA but not in non-OA cats, prompting inclusion of a similar item, Global Distance Examination – General Lameness Score, in the MI-CAT(V)-v4.

Results of the first (gabapentin) and second therapeutic trials (tramadol, OTMS; MI-CAT(V)-v4) permitted further scale refinements based on subscale and individual item performance, resulting in shortening of the scale due to elimination of many individual items, as well as two entire subscales, Standing Up on Hind Feet to Investigate a Higher Surface and Other Behaviors, and condensing of the Gait and Body Posture subcategories, into one single subcategory each. The final version of the scale, the MI-CAT(V)-v5, containing 25 items in 5 subcategories, Body Posture, Gait, Willingness and Ease of Horizontal Movements, Jumping, and Global Distance Examination, was reliable. The MI-CAT(V)-v5 Total Score and all individual subcategories except Willingness and Ease of Horizontal Movements distinguished OA from non-OA cats.

In each of the three therapeutic trials, a treatment effect was detected by objective outcome measures (Klinck, Monteiro et al. 2017, Monteiro, Klinck et al. 2016, Monteiro, Klinck et al. 2017). The scale Total Score did not detect this treatment effect, but some subcategories did. In the gabapentin trial, the MI-CAT(V)-v3's Body Posture – Back, Gait –

Forelimbs, and Standing Up on Hind Feet to Investigate a Higher Surface all improved during treatment in OA, but not in non-OA cats, as did the surgeon's distance (lameness) evaluation. In the second trial, the MI-CAT(V)-v4's Gait and Body Posture – Hind Limbs improved with the combined tramadol and OTMS treatment. In the third and final trial, the MI-CAT(V)-v5's Jumping improved with tramadol treatment, and Willingness and Ease of Horizontal Movements actually worsened. Due to scale modifications between trials, all Body Posture-related items were combined into one category and the same was done for all Gait-related items. There were also item changes (removals, rewording, scoring changes); it is not clear whether apparent inconsistent treatment responsiveness of subcategories was due to chance, or whether scale modifications produced this variability. For instance, although the surgeon's distance (lameness) exam appeared to detect a treatment response in the gabapentin trial, the comparable evaluation included in the MI-CAT(V)-v4 and MI-CAT(V)-v5, Global Distance Examination, did not detect treatment effects in either of these later phases. The latter could suggest that detection of treatment in the first trial was a spurious result, or could be related to unidentified differences in how this item was used as a part of the scale, *vs.* in the surgeon's evaluation, even though attempts were made to ensure that scoring of this item was based on a similar evaluation procedure to that used by the surgeon.

Intra- and inter-rater reliability improved for the MI-CAT(V)-v3 following the major revisions proceeding from the video analysis, and were generally good to excellent for it and for subsequent versions (MI-CAT(V)-v4, MI-CAT(V)-v5). Because inter-rater reliability had suggested an effect of user experience with the scale, in the early studies, more detailed evaluation instructions were included, beginning with the MI-CAT(V)-v3, and a procedure for training novel users was implemented. Improved inter-rater reliability with smaller changes with repeated use suggests that these changes were helpful. However, the MI-CAT(V)-v5's Gait, Jumping, and Body Posture had better intra- than inter-rater reliability, which may indicate that further user training on these items would be helpful. The MI-CAT(V)-v5's Willingness and Ease of Horizontal Movements had better inter- than intra-rater reliability, possibly indicating poor stability over time; poorer results of this subcategory with respect to distinction of OA from non-OA cats, and response to treatment, suggest that item revisions or removal may be warranted. Internal consistency reliability evaluated in the therapeutic trials

supported that scale items were related, without being redundant, particularly when used in the target populations for the scale (cats with and without OA, or with OA). However, similarly to the MI-CAT(C), this result must be interpreted somewhat cautiously due to the large number of scale items (as discussed and below).

3.2.2.3 Contributions to pain management in feline OA

The MI-CAT therapeutic trials involved a variety of analgesics: meloxicam (oral and oral transmucosal spray), tramadol, and gabapentin. Meloxicam is an NSAID, and produces analgesia primarily *via* anti-inflammatory effects. Tramadol is an analgesic with opioid and monoaminergic effects (Beakley, Kaye et al. 2015). Gabapentin is a structural analog of gamma-aminobutyric acid (GABA); however, it appears to exert its effects primarily *via* voltage-gated calcium channel subunit binding (Kukkar, Bali et al. 2013). The latter two medications have some demonstrated efficacy in neuropathic pain (Finnerup, Attal et al. 2015). Objective measures used in the MI-CAT trials detected improvement in cats with OA, in response to every medication/medication combination administered. The MI-CAT(C) also responded to meloxicam treatment (in comparison with placebo), supporting its potential for use in feline OA pain management. However, in addition to the limitations discussed below, it has not been evaluated for its responsiveness to other treatments, such as gabapentin or tramadol. The scale therefore shows promise for the management of feline OA pain (detection of changes in status due to treatment or possibly disease progression), but this must be confirmed with future studies.

The various versions of the MI-CAT(V) were evaluated with each of: meloxicam, gabapentin, tramadol, OTMS, and tramadol with OTMS. Total Score, while able to distinguish OA from non-OA cats in the final version of the scale (MI-CAT(V)-v5), did not detect treatment effects. Ability to detect OA status has potential usefulness for clinical identification of, and implementation of treatment for, cats with OA, but this ability requires confirmation in client-owned cats. Although some scale subcategories showed responsiveness to treatments, this aspect of the scale requires further investigation, as discussed further below. That said, were the MI-CAT(V) able to assist veterinarians in screening at-risk cats for OA, this would benefit management of pain in such cats. A positive (OA) result would prompt further

diagnostics and treatment, and even were the MI-CAT(V) unable to detect response to treatment, owner scales such as the CSOM, or possibly the FMPI or MI-CAT(C), might be used to monitor therapeutic response and disease progression, in order to manage patient pain long-term.

3.2.2.4 Other findings

Although gabapentin has been recommended for treatment of neuropathic and OA pain in cats (Mathews 2008, Rychel 2010), there had been no prior reports describing systematic evaluation of its therapeutic efficacy in feline OA. The scale validation trial involving gabapentin therefore assessed the scale and the OA treatment effects of gabapentin, in parallel. This was possible because of the use of objective outcome measures; treatment efficacy of gabapentin could not be directly evaluated using the unvalidated scale, nor could the scale be directly validated with respect to treatment responsiveness, were there not objective evidence of the treatment's efficacy. Results of objective measures (von Frey-anesthesiometer induced paw withdrawal threshold, and objective activity monitoring) in this study suggested that this drug does provide analgesia in cats with OA and associated central sensitization, supporting this use. However, inferences must be tempered by the fact that there was no placebo group for comparison, and further study is recommended.

3.3 Project limitations and their impact on interpretation of results

There were a number of limitations in this project, some unavoidable, and some due to practical considerations. These are reviewed below, along with their potential influences on the results.

3.3.1 General considerations

Validation of feline OA pain scales is a bit of a catch-22, in that there is no gold standard for the detection of cat OA and associated pain (Epstein, Rodan et al. 2015), but such scales have potential to facilitate diagnosis and disease monitoring. Clinical feline OA detection relies on a combination of radiographic, physical examination, and historical

findings consistent with the presence of the disease (Lascelles and Robertson 2010). However, misclassification of cats is possible during clinical evaluation as well as in selection for any study involving OA and non-OA cats. This risk is due to the mismatch between clinical and structural signs of OA in many species, the difficulties inherent in the feline orthopedic examination, and the subtlety of OA signs observable in the home (Bennett, Zainal Ariffin et al. 2012, Clarke and Bennett 2006, Hansen, Lascelles et al. 2007, Kerwin 2012, Lascelles, Dong et al. 2012), in addition to the possibility that some of the latter signs may not be specific to OA. In this project, a variety of efforts were made to avoid, or to account for, this. The owner survey accepted veterinarians' clinical diagnosis based on a variety of methods of evaluation, but considered what methods were used. In the pilot study and in the first MI-CAT(V) validation study following it, cats were selected for OA and non-OA groups based on the presence of both radiographic and orthopedic examination findings consistent with OA in the same joints, or the absence of OA signs in any joint, respectively (Klinck, Rialland et al. 2015). In the MI-CAT(C) clinical trial, cats were selected based on the presence of both radiographic and orthopedic examination signs of DJD in the same joints, as well as owner-remarked decreases in mobility consistent with DJD (Klinck, Gruen et al. 2017). In the gabapentin trial, the presence (OA cats) or absence (non-OA cats) of allodynia, an indicator of central hypersensitization, were requirements for inclusion, in addition to radiographic findings (OA cats) or the lack thereof (non-OA cats); in the next two trials (involving treatments with tramadol and oral transmucosal meloxicam spray), selection was based on the presence of radiographic OA signs (OA cats) or the absence of both radiographic and orthopedic examination signs consistent with OA (non-OA cats). The choice of the latter inclusion criteria was based on the findings of Guillot et al. 2013, where objective measures of limb function and pain (PVF, PWT) distinguished cats with radiographic signs of OA from those without radiographic signs of OA, and were also responsive to meloxicam treatment in the former cats. In that study, cats diagnosed with OA based on the presence of physical examination signs alone showed neither impairment detectable by PWT and PVF, nor responsiveness to treatment (based on PVF) (Guillot, Moreau et al. 2013).

Determination of rating scale reliability and validity is influenced by sample size; however, no consensus has been established for absolute minimum sample sizes or for

standard procedures for calculation of sample sizes for these purposes (Anthoine, Moret et al. 2014). That said, samples in this project were smaller than those generally recommended and used in the validation of human patient-reported outcomes and proxy scales (Anthoine, Moret et al. 2014, Streiner and Norman 2008). This was due to practical and funding constraints. Small sample sizes could have introduced sampling bias (*i.e.*, the study groups may not have been adequately representative of the general population of cats); in addition, smaller sizes produce larger margins of error, *i.e.*, they increase uncertainty (*e.g.*, larger confidence intervals for reliability estimates). Finally, small samples sizes in these studies precluded the evaluation of internal scale structure using factor analysis. Cronbach's alpha was instead used to assess internal consistency of the scales; however, large numbers of scale items could have artificially increased the values obtained. The overall implication is that the results should be interpreted with caution and require confirmation. However, it is hoped that future studies will be conducted to further evaluate the scales, as described below, thereby expanding the evidence base for their reliability and validity.

3.3.2 Phone survey limitations

Surveys have inherent limitations, and these must be considered in the interpretation of the results. Selection bias was a consideration in the owner survey described here, meaning that the sample may not have been representative of the general population of owners of OA cats. Factors supporting the presence of selection bias in this survey include: 1) that a convenience sample of veterinarians was used to identify OA cat owners for inclusion, 2) that veterinarians selected cases of feline OA from their practices *via* different methods (*e.g.*, records search *vs.* simple recall) that cannot be assumed to have been either all-inclusive or random samplings of clients with OA cats (*i.e.*, some cats with undiagnosed OA were no doubt omitted, and veterinarians may not have identified all cats diagnosed with OA, either by accident or by design), and 3) that the low rate of feline OA diagnosis in these practices suggests that they were a subset of the true population of cats with OA, possibly with particular characteristics making them more likely to be diagnosed. For instance, the high prevalence of gait as a sign could have been due to increased recognition of OA when this sign is present and/or could have been associated with high disease severity (Kerwin 2012). That is,

rather than being due to a high prevalence of gait changes in the overall OA cat population, the study design may have inadvertently selected for OA cats with overt lameness/stiffness; conversely, OA cats without these signs may have been under-represented in the sample. This may also have been the case for other signs of OA. In addition, as noted above, there was no standardized method of diagnosis of OA in the sample; misdiagnosis could have occurred in some cases. The implication of this is that the signs identified in the survey might reflect other causes of pain or musculoskeletal disability. Another consideration is that owners in the sample may have been particularly attentive, making them more likely to observe and to report signs of OA. This cannot be determined based on the study design, but the possibility that some owners may be less attentive or otherwise less able to detect and report signs of OA in their cats should be considered.

In addition to selection bias, surveys are subjective and susceptible to recall bias in respondents. The latter could have contributed to over- or underestimation of certain signs of OA. Furthermore, although owners reported improvements in response to treatment, in many of the reported signs, no conclusions can be drawn regarding treatment responsiveness of signs, due to the survey study design (*e.g.*, subjectivity of assessments, lack of a placebo group, administration of different treatments, *etc.*). There was also a high prevalence of concurrent geriatric diseases in the cats in the survey sample, and it was not possible to distinguish OA signs from those of age-related cognitive or sensory decline, or other geriatric disease. Authors of one study reporting a high concurrence of DJD with CKD suggested that similarity of behavioral signs of CKD to those of DJD (*e.g.*, decreased activity), could have led to attribution of said signs to clinical DJD when in fact they were the result of CKD (Marino, Lascelles et al. 2014). The prevalence of geriatric diseases other than CKD in cats with OA has not been systematically evaluated, and no studies have yet reported attempts to distinguish the signs of OA from those of age-related cognitive or sensory decline, or other geriatric diseases; the extent of overlap of signs is therefore unknown.

3.3.3 Scale development and validation study limitations

The MI-CAT(C) and MI-CAT(V) development and validation procedures were based on recommendations in the literature. However, some aspects either were not addressed in this project, or could have been handled differently.

3.3.3.1 Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner

With respect to scale development, the owner survey was conducted in parallel with the development and validation of content, rather than prior to development. However, since the results generally provided support for the MI-CAT(C)'s content, it is not clear that performing the owner survey earlier in the scale development process would have provided particular benefit.

Comprehensibility of the MI-CAT(C) was assessed by asking cat owners in the clinical trial to rate the clarity of items. However, it was not otherwise systematically evaluated. Although comprehension was found to be good for most items, it would have been desirable to evaluate readability formally, and to perform an in-depth assessment of comprehensibility (see below). Indeed, it is possible that the owners participating in the clinical trial were not representative of the general population of cat owners (*e.g.*, comprehension might be poorer in other samples of cat owners).

Regarding validation of the MI-CAT(C), it would have been preferable to conduct all evaluations in client-owned cats, rather than using a laboratory colony for preliminary validation and reliability testing (pilot study). However, it was recognized during this phase that results required cautious interpretation because of differences in the sample from the target population, and limited modifications were made to the scale on this basis. The pilot study provided the only comparison of scale results between OA and non-OA cats; ability to detect OA status in a client-owned sample of cats has not been assessed. This is pertinent in the consideration of whether the scale truly measures OA pain, or whether it may be influenced by other (undetected in the clinical trial sample) meloxicam-responsive conditions. The potential influence on the MI-CAT(C) of nonspecific age-related changes, as well as age-

related cognitive or sensory decline, or geriatric diseases other than OA, has not been evaluated (evidence of validity based on relations to other variables; discriminant validity).

3.3.3.2 Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians

Similarly to the MI-CAT(C), the MI-CAT(V)'s comprehensibility was assessed by asking potential respondents (third-year veterinary students) to rate the clarity of items, and was found to be good generally. A veterinarian unfamiliar with the MI-CAT(V) also reviewed and commented on it. However, no further evaluation of comprehensibility or readability was performed. Although the average reading level of veterinarians may be expected to be higher than that of cat owners (due to level of education), this is an aspect of the MI-CAT(V) that should be examined.

With respect to scale development, it could be argued that the video analysis would have been better conducted prior to scale development and preliminary validation, as it was used to inform scale content (*i.e.*, to generate items, response options, and scale evaluation procedure). However, 1) the process initially used for content development was sound (literature review and expert opinion) and expert review supported content validity, and 2) preliminary evaluation in the context of the pilot study permitted the video analysis to target subcategories of the MI-CAT(V) that seemed promising, potentially improving results by limiting participant fatigue.

Rating scales require evaluation under conditions comparable to those of their targeted use (*i.e.*, with respect to subjects, context, and evaluators). The MI-CAT(V) has thus far only been tested in laboratory cats. Consequently, this project provides support for its validity and reliability in laboratory cats with naturally-occurring OA. It may therefore be employed in screening such cats for OA, but this is of limited usefulness in laboratory populations of cats, without evidence of therapeutic responsiveness. The latter would permit scale use to assess the therapeutic efficacy of OA treatments in a laboratory research setting. The scale's value as a screening tool for OA would arguably be greater in client-owned cats, in clinical practice, but its validity and reliability in this population will require confirmation *via* study in samples of such cats.

Other limitations in the validation of the MI-CAT(V) include the lack of a placebo group in the first (gabapentin) trial and the use of many of the same cats in the second and third therapeutic trials (despite blinding of evaluators). The former, combined with the observation that both OA and non-OA cats treated with gabapentin showed improvement in objective measures (night-time activity, statistically significant for both groups; paw withdrawal threshold, statistically significant for OA cats but not for non-OA cats), mean that apparent treatment effects on the subjective outcome measures in the trial should be interpreted with caution. Re-use of subjects from one trial to another (following modifications to the scale) could have selected for characteristics particular to the sample of cats, particularly given the small sample size. In addition, like the MI-CAT(C), the MI-CAT(V) has not been evaluated for its ability to discriminate OA from the effects of nonspecific age-related changes, cognitive or sensory decline, or other geriatric diseases (evidence of validity based on relations to other variables; discriminant validity).

Finally, although the MI-CAT(V) Total Score and the scores of subcategories were assessed for their ability to detect treatment effects, in each therapeutic trial, individual item analysis of validity focused primarily on distinction of OA from non-OA cats. It is not known whether this emphasis could have led to item changes that decreased scale responsiveness to treatment (*i.e.*, it is possible that different MI-CAT(V) items detect OA disease status and treatment-related changes in pain).

3.3.3.3 Solutions to study limitations

Some of the limitations described above could have been addressed *via* different methodology. For instance, the difficulty of assigning OA status to cats, due to the lack of a gold standard for diagnosis, has no perfect solution. However, an ideal method of screening would assess all joints for structural changes and assess the cat for evidence of pain or functional changes shown to be associated with OA. Because radiography detects bony, but not soft tissue changes, it could be of value to implement an imaging modality capable of detecting both bone and cartilage changes, such as MRI. While optimal for confirming the presence/absence of OA-related joint lesions, this would be difficult/impractical due to the costs, anesthetic time and risks, *etc.*, associated with an MRI procedure. However, additional

functional screening that could be performed to rule in/out OA could include: determination of paw withdrawal threshold (von Frey) or evaluation of PVF using a force plate or pressure-sensing walkway (Addison and Clements 2017, Guillot, Moreau et al. 2013), in addition to owner reports of reduced mobility.

Sample size limitations due to practical considerations (costs, space, personnel, difficulties of recruiting non-OA cats) could potentially be addressed by combining MI-CAT(C) or MI-CAT(V) data from several study groups. This would of course require that the same scale version be used in all studies, and screening methods for OA should be homogeneous across groups. It could potentially be accomplished *via* collaborations with other research groups studying feline OA, essentially to "piggyback" MI-CAT(C) or MI-CAT(V) completion onto their studies. This would permit an evaluation of scale reliability in a larger sample (ideally, greater than 100 cat-owner pairs for the MI-CAT(C), or cats for the MI-CAT(V)). Another possibility would be to recruit cases from a clinical population (*e.g.*, multiple private practice veterinarians), which would require standardization of diagnostic procedures (*e.g.*, radiographic, historical and physical examinations) in the home clinics, or referral to one or more tertiary centre(s) for the diagnostic procedures.

Selection bias in the phone survey could have been minimized by ensuring a more random sample of cats, with standardization of diagnostic methods. For instance, in the initial development of the FMPI (Zamprogno, Hansen et al. 2010), cats were randomly selected for OA screening, from the patient database of a private veterinary practice. Although the selected clinic might have an effect on the client-cat sample, and the owners' willingness to participate would introduce some selection bias, this is one way to attempt to reduce such bias. Other limitations of our phone survey, such as recall bias and the presence of comorbidities in many cases, could have been minimized by also interviewing owners at the time of diagnosis (ideally, prior to treatment), and by screening all cats for concurrent disease and retaining only those with OA alone.

With respect to the development of the MI-CAT(C), it would have been appropriate to conduct a more thorough assessment of respondent burden (Lohr 2002), particularly scale comprehension. Respondents in the clinical trial (section 2.2) were asked whether each scale

item was clear or not, information on missing scale responses was reviewed, and it was determined that primary cat owners rather than other household members should complete the scale (*i.e.*, that owner familiarity with the cat influences the scale outcomes). However, additional measures that should have been assessed include: information on the time to complete the scale, an evaluation of readability using one or several available scoring systems (*e.g.*, Flesch Readability Score, Gunning Fog Index) (Flesch 1948, Klare 1974), and determination of scale comprehensibility (*e.g.*, *via* cognitive interviews) (Collins 2003, Patrick, Burke et al. 2011).

Initial pilot testing of the MI-CAT(C) in a small group of laboratory cats meant that the results of the latter study required cautious interpretation. A solution to this limitation would have been to have pilot tested the scale in a larger sample of client-owned OA and non-OA cats, possibly by piggy-backing onto an existing study in such animals (requiring collaboration with outside researchers). In addition, testing the MI-CAT(C) in client-owned cats with and without OA would have provided information on the scale's ability to distinguish the two groups, and could have guided the establishment of scale score thresholds for OA diagnosis.

As noted above, in the development of the MI-CAT(V), retention of items based on distinction of OA *vs.* non-OA status may have led to rejection or modification of items that could be sensitive to OA treatment. The final version of the scale did not detect response to the treatment with an analgesic, despite detectable improvements in outcome measures. In addition to further refinement of existing items, it may be of value to re-examine eliminated items for their treatment responsiveness. If items were identified with this attribute, they might be considered for a scale version to be used specifically to assess treatment effects. Other limitations, such as the lack of assessment of the scale in client-owned cats, the lack of an evaluation of respondent burden, the need for testing of discriminant validity, and the need to confirm that the ability to distinguish OA from non-OA cats will be retained in other groups of cats, will need to be assessed in future studies (as described for the MI-CAT(C), above, and as discussed further, below).

3.4 Future research directions

Although this project provides evidence supporting the validity and reliability of the MI-CAT(C) and the MI-CAT(V), both scales require further work. Recommendations for each are described below.

3.4.1 The Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner: further validation

The MI-CAT(C) detected treatment, but has not been evaluated for its ability to discriminate OA and non-OA cats. Scale score differences between the latter two groups may be greater than those resulting from treatment, in OA cats; for instance, the MI-CAT(V) distinguished OA from non-OA cats but did not detect treatment effects, and the same was the case for an early version of the FMPI (Benito, Depuy et al. 2013, Benito, Hansen et al. 2013, Klinck, Monteiro et al. 2017). However, the MI-CAT(C) cannot be assumed to detect OA status, without evidence based on testing in a sample of OA and non-OA cats. Such an evaluation would also help to confirm that the scale indeed measures OA, rather than other meloxicam-responsive conditions (*e.g.*, that may have been undetected in the clinical trial). In this vein, it would also be appropriate for the scale to be assessed for discriminant validity, *i.e.*, its relations with other age-related disorders (*e.g.*, sensory and cognitive decline, and common geriatric medical diseases such as diabetes mellitus, CKD, cardiac disease, and hyperthyroidism), particularly given the possibility of a high prevalence of comorbidities in OA cats (Klinck, Frank et al. 2012, Marino, Lascelles et al. 2014). Finally, some modifications to the MI-CAT(C) were suggested by the results of the clinical trial of meloxicam described here; the revised scale requires reassessment to support its reliability and validity in assessing treatment effects in OA cats.

Feline OA pain scales have potential both in research (evaluation of new therapeutic agents) and in a clinical context (to facilitate diagnosis and monitoring of pain and disability in individual cases of feline OA). The MI-CAT(C) will require further study in addition to confirmation that it measures OA, as discussed above. First, a systematic evaluation of scale

comprehension, particularly of readability, is recommended. Second, distinction of treatment from placebo effect has been variable for other feline OA owner scales (Gruen, Griffith et al. 2015, Gruen, Thomson et al. 2016, Lascelles, Hansen et al. 2007) and further research should confirm the MI-CAT(C)'s ability to do so. Related to this, the scale has only been tested for therapeutic responsiveness with meloxicam; its ability to detect the effects of other treatments should be investigated. Third, particularly for it to be used for clinical decision-making, cut-points will need to be established (*e.g.*, for detection of treatment efficacy or worsening disease, in individual animals). Statistically significant changes in scores do not necessarily equate to clinically significant changes; the minimally important change (MIC; or minimum (clinically) important difference, MID or MCID) is defined as the minimum change on a rating scale that reflects a meaningful change in patient status, and may be assessed on the basis of the response distribution or on the basis of an external anchor (*e.g.*, in humans, self-reported meaningful change); distribution based methods have been argued to reflect the minimally detectable change (MDC), rather than the MIC (de Vet, Terwee et al. 2006). The MDC is related to (and evidently sometimes conflated with) the MIC, and refers to the smallest change that falls outside the measurement error of the instrument (de Vet, Terwee et al. 2006), and may often approximate 0.5 standard deviations (Streiner and Norman 2008). Determination of the MDC and MIC in scores would be helpful to guide veterinarians' use of the MI-CAT(C) for making therapeutic decisions, and could facilitate interpretation of research results using the scale. To establish an MIC, a common method in human medicine is to compare scale score changes with patient or clinician global ratings of improvement/deterioration in condition, and to determine the minimum score change associated with a change in the global rating (Crosby, Kolotkin et al. 2003). Hence, in cats, a potential method of determining MIC would be to compare MI-CAT(C) scores with clinician or owner global ratings (*i.e.*, external anchors); this is evidently an imperfect method, given the difficulties inherent in human assessment of feline OA pain. The determination of MDC might be more feasible; this could be accomplished, for instance, using a formula based on the standard error of the mean or on the standard error of measurement difference (using pre- and post-treatment scores) (Crosby, Kolotkin et al. 2003). In addition, if the scale is found to distinguish OA from non-OA cats, thresholds for classification of OA *vs.* non-OA should be established (*e.g.*, through the use of receiver operating characteristic curves) (Streiner and

Norman 2008). Fourth, feasibility (*i.e.*, ease of use in the target context) and clinical utility (*i.e.*, how use of the scale contributes to case management) of the scale should be investigated (Duhn and Medves 2004, Gélinas 2010). A fifth potential area of study is whether results with the MI-CAT(C) differ for different subsets of cat owners, *i.e.*, are there particular characteristics of owners that influence the reliability and validity of scale use. This is particularly pertinent since scoring varied for different household members, in the clinical trial (Klinck, Gruen et al. 2017). It was inferred that the primary owner should complete the scale, but even primary owners (as proxies, and therefore a source of error) may differ in the validity and reliability of their reporting of feline OA signs.

3.4.2 The Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians: further refinement and validation

The MI-CAT(V) detected naturally-occurring OA in laboratory cats, in a research setting, but it has not yet been evaluated in client-owned cats in a clinical practice setting. It did not detect therapeutic efficacy demonstrable with objective outcome measures (objective activity monitoring, von Frey anesthesiometer-induced paw withdrawal threshold, and response to mechanical temporal summation). While a feline OA pain scale may be of use in a research laboratory setting, such usefulness is limited in the absence of treatment responsiveness. Conversely, a tool capable of screening for OA in cats in a clinical setting could arguably be of value, even without the ability to detect treatment effects. Therefore, the MI-CAT(V) requires evidence of reliability and validity in client-owned cats, in a clinical setting, and/or refinement to permit detection of treatment in OA cats (in a laboratory and/or clinical setting), to fulfill one or the other, or both, of these purposes. It is not known whether therapeutic responsiveness can be attained; criteria that detect the presence of OA may differ from those that detect changes in OA pain status. However, given the apparent treatment responsiveness of some scale items/subcategories in the therapeutic trials described here, this may be achievable. Potential strategies to improve sensitivity to OA treatment could include: weighting of promising items, or possibly devising alternate forms of the MI-CAT(V) for detection and for treatment monitoring. This might be accomplished *via* re-evaluation of items previously discarded for poor distinction of OA *vs.* non-OA cats, or through the use of a subset

of the current scale, *e.g.*, including such promising subcategories as Gait, Jumping, and possibly Posture.

As discussed for the MI-CAT(C), the MI-CAT(V) could benefit from a systematic evaluation of comprehensibility, especially readability. Establishment of cut-points would also be needed, particularly for clinical use (*e.g.*, determination of thresholds for categorization of at-risk cats as OA *vs.* non-OA). An important aspect of testing the MI-CAT(V) in client-owned cats, in a clinic setting, will be the assessment of feasibility, as there may be challenges in implementing the evaluation procedure. Scale length and complexity, as well as time to complete assessments and scoring, influence feasibility; however, reliability of the scale appeared to decrease when the number of items was reduced. Hence, further scale refinements for ease of use must be balanced against the need for reliability. Determination of the clinical utility of the scale would also be helpful. In addition, effects of user training for this scale could be further investigated to determine: 1) what training procedure(s) optimize(s) scale outcomes (*e.g.*, for items with weaker or inconsistent inter-rater reliability such as Gait, Posture, and Jumping) and feasibility, and 2) whether and at what intervals training may need to be repeated.

Finally, systematic comparisons between the available owner scales (*e.g.*, the FMPI, CSOM, Bennett and Morton scale, and the MI-CAT(C)) and the MI-CAT(V) could be performed. This would permit an evaluation of areas of convergence and divergence (validity based on evidence of relations with other variables; construct validity), *i.e.*, whether they measure different aspects of feline OA. In addition, such comparisons could provide information helpful for selecting a particular pain scale for use, in a given clinical or research context.

3.5 Conclusion

Clinical feline OA, while it has received increasing interest in recent years, remains challenging to diagnose and to monitor. The research reported here forms a significant contribution to the literature on this topic.

Pain scales are an attractive method for providing some measure of standardization in subjective pain assessments, both by pet owners and by veterinarians. The development and validation processes for the MI-CAT(V) and MI-CAT(C), that formed this project, have provided an initial body of evidence supporting the potential of both of these tools. In particular, the MI-CAT(C) was found to distinguish meloxicam treatment from placebo in cats with OA, and the MI-CAT(V) was able to differentiate OA from non-OA cats. This is an exciting advance that is hoped to contribute to the detection, measurement, and management of the disease. The MI-CAT(C) adds to the existing owner pain scales for feline OA. In addition, findings of the clinical trial suggest that owner-cat relationships may influence scale outcomes; this may have broader implications for the use of owner report/pain scales in detecting/assessing feline OA. The MI-CAT(V) is the first scale reported for use by veterinarians; this research also underscored limitations of the traditional use of joint palpation and manipulation for evaluating feline OA. Because joint palpation and manipulation tend to suffer from poor sensitivity for feline OA, a tool augmenting the orthopedic examination of at-risk cats would be a boon to veterinarians.

However, just as other described tools are imperfect at this time, the MI-CAT scales require additional work both to support their use in their current forms, and potentially to expand their use. Limitations of this research include the lack of assessment of the MI-CAT(C) in OA *vs.* non-OA cats, the need to evaluate the MI-CAT(V) in client-owned cats, the small sample sizes, the lack of comprehension and readability analyses for the scales, and the potential for selection bias in the phone survey and the MI-CAT(C) clinical trial. Further work on the MI-CAT scales is needed, particularly to establish guidelines for clinical use (including the establishment of cut-points for clinical decision-making using either scale, and of a standardized training procedure for the MI-CAT(V)). In addition, confirmation of the ability of both scales to distinguish OA from non-OA status in client-owned cats, and that the MI-CAT(C) detects changes in disease status (*e.g.*, in response to various treatments), is needed. The MI-CAT(V) also requires refinement and further evaluation to establish whether it may eventually be used to monitor changes in disease status. The work described in this Thesis should provide a jumping off point for other researchers to reproduce these findings and to advance the development and validation process for the MI-CAT scales.

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5. APPENDICES

5.1 Appendix A

5.1.1 Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – v1

MI-CAT(C)-v1 – Montreal Instrument for Cat Arthritis Testing (Caretaker) – Version 1

Instructions:

Check all items in both tables that describe your cat as he is NOW. Add the number of checks for each table separately.

Table 1			
Category	#	Item	*Circle Answer* **Y**=Yes **N**=No **U**=Don't Know/Does not Apply
Agility	1	My cat moves smoothly and gracefully	Y N U
	2	My cat can jump UP 3 feet or more to reach high places to rest in, play on, or investigate	Y N U
	3	My cat can jump DOWN 3 feet or more	Y N U
	4	My cat easily runs UP stairs	Y N U
	5	My cat easily runs DOWN stairs	Y N U
	6	My cat runs on flat surfaces	Y N U
	7	My cat climbs vertical surfaces (such as a cat tower/furniture/trees)	Y N U
	8	My cat leaps into midair when playing	Y N U
Social, Play, and Exploratory Behaviors	9	My cat plays on their own (indoors) OR hunts mice and other wildlife (if allowed outdoors)	Y N U
	10	My cat wants to play with family members/other pets	Y N U
	11	My cat sneaks/tries to sneak through doors to get outside or into off-limits parts of the house	Y N U
	12	My cat investigates and plays with new objects or furniture	Y N U
	13	My cat seeks and finds objects to play with	Y N U
	14	My cat steals/attempts to steal people food or other food that isn't in a pet dish	Y N U
	15	My cat follows family members around the house	Y N U
	16	My cat enjoys being petted by people or groomed by other pets	Y N U
	17	My cat enjoys being picked up or held	Y N U
	18	My cat lies on or against family members or other pets	Y N U
	19	My cat greets family members when they wake up or come home	Y N U
	20	My cat grooms/licks other pets or family members	Y N U

MI-CAT(C)-v1 (continued)

Self-Maintenance	21	My cat digs vigorously in the litter box, or in the dirt outdoors	Y N U
	22	My cat bolts out of the litter box	Y N U
	23	My cat can groom their whole body easily	Y N U
	24	My cat stretches up to sharpen their claws on, or to paw at (if declawed), vertical surfaces	Y N U
	25	My cat sharpens their claws on, or paws at, horizontal surfaces	Y N U
	26	My cat regularly stretches by extending both his front forward and then both his back feet out behind him	Y N U
	27	My cat can easily scratch their head or neck with either hind foot	Y N U
Total Score for Table 1 (Count "Yes" Responses)			

Table 2			
Category	#	Item	*Circle Answer* **Y**=Yes **N**=No **U**=Don't Know/Does not Apply
Agility	1	My cat is clumsy or awkward in their movements	Y N U
	2	My cat moves stiffly or limps at times	Y N U
	3	Instead of walking or running normally, my cat sometimes "bunny hops" with both back or front feet together	Y N U
	4	My cat has one or more limbs that trembles at times	Y N U
	5	My cat seems to hesitate or to avoid jumping UP	Y N U
	6	My cat seems to hesitate or to avoid jumping DOWN	Y N U
	7	My cat often misses when trying to jump (scrambles or stumbles)	Y N U
	8	My cat can't/won't jump UP more than 1-1½ feet	Y N U
	9	My cat can't/won't jump DOWN more than 1-1½ feet	Y N U
	10	My cat prefers several small hops to one bigger jump UP	Y N U
	11	My cat prefers several small hops to one bigger jump DOWN	Y N U
	12	My cat hesitates/pauses going UP stairs or takes them slowly/one at a time	Y N U
	13	My cat hesitates/pauses going DOWN stairs or takes them slowly/one at a time	Y N U
	14	My cat sits, lies down, or gets up slowly or stiffly	Y N U
	15	My cat asks to be lifted or carried up/down the stairs or to/from elevated locations (e.g., window, table, bed)	Y N U
	16	My cat's footsteps sound uneven	Y N U

MI-CAT(C)-v1 (continued)

Social and Play Behaviors	17	My cat doesn't like to play	Y N U
	18	My cat tends to be out of sight or keeps to themself	Y N U
	19	My cat leaves the area when any family member or other pet approaches them while they are resting	Y N U
	20	My cat sleeps almost all the time that family members are home	Y N U
Self-Maintenance	21	My cat urinates and defecates in the house, away from the litter box	Y N U
	22	My cat has trouble getting in/out of the litter box, or gets urine or stool just outside the box	Y N U
	23	My cat's fur seems dull/flaky/untidy	Y N U
	24	My cat doesn't wash themself well or often	Y N U
	25	My cat has ungroomed areas or mats	Y N U
	26	My cat's claws are long/dull, or get caught in things *(leave blank if declawed on all four feet)*	Y N U
	27	My cat doesn't come right away for food or treats	Y N U
Physical Condition	28	If I had to rate my cat's body condition (shape) on the following scale, it would be a 4 or 5: (see attached diagrams 1-5 BCS)	Y N U
	29	My cat has a crouched appearance of his front legs when he is standing (see Photo 1)	Y N U
	30	My cat has a crouched appearance of his rear legs when he is standing (see Photo 1)	Y N U
	31	My cat sometimes looks hunched when he is standing or walking (see Photo 2 (normal posture) and Photo 3 (hunched back))	Y N U
	32	My cat sometimes has their weight shifted off one (or more) particular legs or puts a leg out to the side when walking, standing, sitting, or lying (see Photo 4 (normal, symmetrical sitting posture), Photo 5 (normal lying posture), Photo 6 (standing with one front paw held to side))	Y N U
Total Score for Table 2 (Count "No" Responses)			

5.1.2 Montreal Instrument for Cat Arthritis Testing, for use by Caretaker/Owner – v2

MI-CAT(C)-v2 – Montreal Instrument for Cat Arthritis Testing (Caretaker) – Version 2

Instructions:

Please indicate the name of the person completing the questionnaire, the cat's name, and the date at the top of each page.

Check all items in both tables that describe your cat as he is NOW. Add the number of checks for each table separately.

Table 1			
Category	#	Item	*Circle Answer* **Y**=Yes **N**=No **U**=Don't Know/Does not Apply
Agility	1	My cat moves smoothly and gracefully	Y N U
	2	*My cat can jump UP 3 feet or more to reach high places to rest in, play on, or investigate*	*Y N U*
	3	My cat can jump DOWN 3 feet or more	Y N U
	4	My cat easily runs UP stairs	Y N U
	5	My cat easily runs DOWN stairs	Y N U
	6	My cat runs on flat surfaces	Y N U
	7	*My cat climbs vertical surfaces (such as a cat tower/furniture/trees)*	*Y N U*
	8	My cat leaps into midair when playing	Y N U
Social, Play, and Exploratory Behaviors	9	My cat wants to play with family members/other pets	Y N U
	10	My cat sneaks/tries to sneak through doors to get outside or into off-limits parts of the house	Y N U
	11	*My cat steals/attempts to steal people food or other food that isn't in a pet dish*	*Y N U*
	12	My cat enjoys being picked up or held	Y N U
	13	My cat lies on or against family members or other pets	Y N U
Self-Maintenance	14	My cat can groom their whole body easily	Y N U
	15	My cat stretches up to sharpen their claws on, or to paw at (if declawed), vertical surfaces	Y N U
	16	*My cat sharpens their claws on, or paws at, horizontal surfaces*	*Y N U*
	17	My cat regularly stretches by extending both front feet forward and then both back feet out behind	Y N U
	18	My cat can easily scratch their head or neck with either hind foot	Y N U
A	Table 1 total number of "Yes" responses:		
B	Table 1 total number of "No" responses:		

MI-CAT(C)-v2 (continued)

Table 2			
Category	#	Item	*Circle Answer* **Y**=Yes **N**=No **U**=Don't Know/Does not Apply
Agility	1	My cat moves clumsily or awkwardly	Y N U
	2	My cat moves stiffly or limps	Y N U
	3	My cat gets up stiffly after resting	Y N U
	4	My cat seems to hesitate or to avoid jumping UP	Y N U
	5	*My cat seems to hesitate or to avoid jumping DOWN*	*Y N U*
	6	**My cat can't/won't jump UP more than 1-1½ feet**	**Y N U**
	7	**My cat can't/won't jump DOWN more than 1-1½ feet**	**Y N U**
	8	*My cat prefers several small hops to one bigger jump UP*	*Y N U*
	9	My cat prefers several small hops to one bigger jump DOWN	Y N U
	10	My cat hesitates/pauses going UP stairs or takes them slowly/one at a time	Y N U
	11	My cat hesitates/pauses going DOWN stairs or takes them slowly/one at a time	Y N U
	12	My cat asks to be lifted or carried up/down the stairs or to/from elevated locations (e.g., window, table, bed)	Y N U
	13	***My cat doesn't like to play***	***Y N U***
Self-Maintenance	14	My cat has trouble getting to/into, or out of the litter box, or gets urine or stool outside the box	Y N U
	15	My cat's fur seems dull/flaky/untidy	Y N U
	16	My cat doesn't wash themself well or often	Y N U
	17	**My cat's claws are long/dull, or get caught in things (leave blank if declawed on all four feet)**	**Y N U**
Physical Condition	***18***	***If I had to rate my cat's body condition (shape) on the following scale, it would be a 4 or 5: (see attached diagrams 1-5 BCS)***	***Y N U***
	19	**My cat looks hunched or crouched when standing or walking (see Photo 1 (normal posture) and Photos 2 (hunched back) and 3 (crouched))**	**Y N U**
	20	***My cat tends to have their weight shifted off one (or more) particular legs or puts a leg out to the side when walking, standing, sitting, or lying (see Photo 4 (normal, symmetrical sitting posture), Photo 5 (normal lying posture), Photo 6 (standing with one front paw held up), Photo 7 (standing with one front paw held to side)***	***Y N U***
C	Table 2 total number of "Yes" responses:		
D	Table 2 total number of "No" responses:		

Legend: Items with poorer owner comprehension are in bold. Those with item-subscale total correlations < 0.20 on Days 0 and 15 are indicated in italics.

5.2 Appendix B

5.2.1 Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians – v1

MI-CAT(V)-v1 – Montreal Instrument for Cat Arthritis Testing (Veterinary) – Version 1

#	Category	Assessment Criteria	Grade
Assign a value for each of categories 1-4 prior to hands-on examination. The cat should be allowed to walk on exam room floor, be placed on a low bench or chair to observe willingness/ability to jump down, and encouraged to jump up by placing the empty carrier on a bench/chair in front of the cat.			
1	Exploratory Behavior	Walks, runs or jumps freely	0
		Walks slowly/cautiously, or with abnormal or lowered body posture	1
		No ambulation/exploratory behavior	2
2	a. Body Posture – **head, torso, tail**	Ambulates/stands/sits/lies with even weight distribution from front to rear, back level, head up, tail above horizontal	0
		Head low/tail lowered (not tucked)	1
		Overt abnormalities: weight shifted forward or backward, hunched back, limp tail - 1 finding	2
		- ≥2 findings	3
	b. Body Posture – **front limbs**	Ambulates/stands/sits/lies with limbs in normal state of flexion/extension, even weight distribution from right to left	0
		Overt abnormalities: limb hyperflexion, limb hyperextension, unequal weight distribution from right to left, or other asymmetry - 1 finding	1
		- ≥2 findings	2
	c. Body Posture – **rear limbs**	Ambulates/stands/sits/lies with limbs in normal state of flexion/extension, even weight distribution from right to left	0
		Overt abnormalities: plantigrade stance, limb hyperflexion or hyperextension, unequal weight distribution from right to left, or other asymmetry - 1 finding	1
		- ≥2 findings	2
3	Gait/Locomotion	Normal gait, jumps up/down willingly and smoothly	0
		Normal gait, reluctant or unwilling to jump	1
		Generally normal gait, occasionally awkward (e.g., misses a jump or missteps)	2
		Mildly to moderately abnormal gait (e.g., stiff or weak, or with abnormal limb placement or carriage)	3
		Obviously limping on 1 or more limbs	4

MI-CAT(V)-v1 (continued)

4	Interaction with Examiner	Friendly: approaches/rubs/wants to be petted	0
		Immobile: neither avoids nor solicits contact	1
		Withdraws/avoids touch	2
		Hisses/growls/swats/bites or threatens	3
5	Body Condition Score	Normal: 3/5	0
		Thin: 1/2-2/5	1
		Overweight: 4/5	1
		Obese: 5/5	2
6	a. Coat Condition	Clean, shiny, no mats	0
		Unkempt, flaky, not shiny; no overt dirt/mats	1
		Dirty/matted (localized)	2
		Dirty/matted (generalized)	3
	b. Claw Condition Note overgrown claws (not abnormal claws in polydactyls). Note "0" if all feet declawed.	Claws sharp (unless trimmed), normal length/thickness	0
		Occasional overgrown (thick or excessively long) claw	1
		Most claws on one or more paws overgrown	2

For categories 7 and 8, perform palpation and manipulation (including full flexion/extension, as well as assessment of side-to-side and cranial-caudal motion) of the neck, back, tail, and each appendicular joint, with the patient standing first, then in lateral recumbency. Do not repeat manipulation if patient attempts to bite.

7 Palpation and Manipulation – **Findings** (check ALL that apply)

Joint	Muscle atrophy	Joint thickening	Crepitus	Reduced range of motion	Total for each joint (out of 4)
Cervical spine					/4
Thoracolumbar spine					/4
Sacrococcygeal spine					/4
Manus					/4
Carpus					/4
Elbow					/4
Shoulder					/4
Pes					/4
Tarsus					/4
Stifle					/4
Hip					/4

MI-CAT(V)-v1 (continued)

8	Palpation and Manipulation – **Cat Response** (check ALL that apply)					
Joint		Flinches/ withdraws	Vocalizes /hisses/ growls	Threatens to bite or scratch	Response is repeatable	Non-evaluable response (ex: aggression)
Cervical spine						
Thoracolumbar spine						
Sacrococcygeal spine						
Manus						
Carpus						
Elbow						
Shoulder						
Pes						
Tarsus						
Stifle						
Hip						

5.2.2 Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians – v2

MI-CAT(V)-v2 – Montreal Instrument for Cat Arthritis Testing (Veterinary) – Version 2

#	Assessment Criteria		Grade
Assign a value for each of categories 1-4 prior to hands-on examination. The cat should be allowed to walk on exam room floor, be placed on a low bench or chair to observe willingness/ability to jump down, and encouraged to jump up by placing the empty carrier on a bench/chair in front of the cat.			
1	*Exploratory behavior*		
	Walks and/or runs; jumps freely		0
	Walks and runs; jumps with mild encouragement		1
	Walks but does not run; jumps with mild encouragement		2
	Walks and runs; reluctant or unwilling to jump		3
	Walks normally; does not run; reluctant or unwilling to jump		4
	Walks slowly/cautiously, or with abnormal or lowered body posture		5
	Recumbent/sitting (no ambulation)		6
2	*Gait*		
	Normal gait		0
	Not assessable – limited or no ambulation		1
	Occasionally awkward (e.g., misses a jump or missteps)		2
	Mild abnormality (e.g. inconsistent/mild stiffness/weakness or abnormal limb placement/carriage)		3
	Moderate abnormality (e.g. consistent/moderate stiffness/weakness or abnormal limb placement/carriage)		4
	Obviously limping on 1 or more limbs		5
3	*Body Posture*		
	a. head, torso, tail	Ambulates/stands/sits/lies with even weight distribution from front to rear, back level, head up, tail above horizontal	0
		Head low/tail lowered (not tucked)	1
		Overt abnormalities: weight shifted forward or backward, hunched back, limp tail 1 finding	2
		≥2 findings	3
	b. front limbs	Ambulates/stands/sits/lies with limbs in normal state of flexion/extension, even weight distribution from right to left	0
		Overt abnormalities: limb hyperflexion, limb hyperextension, unequal weight distribution from right to left, or other asymmetry 1 finding	1
		≥2 findings	2
	c. rear limbs	Ambulates/stands/sits/lies with limbs in normal state of flexion/extension, even weight distribution from right to left	0
		Overt abnormalities: plantigrade stance, limb hyperflexion or hyperextension, unequal weight distribution from right to left, or other asymmetry 1 finding	1
		≥2 findings	2

MI-CAT(V)-v2 (continued)

4	*Interactions with examiner*		
	Friendly: approaches/rubs/wants to be petted		0
	Neither avoids nor solicits contact		1
	Withdraws/avoids touch		2
	Hisses/growls/swats/bites or threatens		3
Perform palpation and manipulation in whatever position is best tolerated by the patient. Assess whether response is present and repeatable, and association with pain. A response may consist of: tensing, flinching, withdrawing or attempting to escape, vocalization (hiss, growl, meow, etc.), turning toward the handled body part or threatening to bite or scratch, or biting or scratching.			
5	*Cat response to palpation and manipulation*		
	a. cervical spine	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	b. thoracic spine	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	c. lumbar (and lumbosacral) spine	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	Total score: axial skeleton		/6
	d. carpus	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	e. elbow	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	f. shoulder	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	Total score: front limb		/6
	g. tarsus	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	h. stifle	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	i. hip	No repeatable response	0
		Repeatable response, but not clear if due to pain	1
		Repeatable painful response	2
	Total score: rear limb		/6

Add scores for 1, 2, 3a-c, 4, 5 Total axial skeleton, Total front limb, Total rear limb	
Total MI-CAT(V) score	**/ 39**

5.3 Appendix C

5.3.1 Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians – v3

MI-CAT(V)-v3 – Montreal Instrument for Cat Arthritis Testing (Veterinary) – Version 3

#	Assessment Criteria	Response Options			Grade
General Instructions • *Perform evaluation via distance observation, with the cat on the examination room floor.* • *To stimulate locomotion, use only things the cat is likely to want/enjoy; that is, toss treats or toys (e.g., cotton ball) for it to chase, or call, gesture enticingly, or pet the cat to encourage approach.* • *DO NOT physically push or pull the cat, guide it by a leash or collar, or startle it to stimulate locomotion (this will interfere with natural movements).* • *Complete criteria in listed order, or in order of convenience based on unsolicited movements performed by the cat. However, the cat should only be encouraged to investigate the elevated surface and to jump up (Criteria 8 and 9) after assessment of Criteria 1-7 is complete.* • *<u>Please note:</u> although each listed criterion has the potential to be expressed in affected cats, it is not expected that every criterion will be present in a single animal.*					
Evaluation Procedure *1)Place cat on floor and observe undisturbed behavior, appearance, posture and movements.* *2) Encourage cat (see above) to walk, run, turn, etc., so that gait can be observed from the side and from behind/in front, and so that willingness to move about can be assessed.* *3) Encourage cat (see above) to investigate an elevated horizontal surface, e.g., bench, chair, shelf, ~15-24" tall. If the cat neither jumps up, nor places its front paws up, it can be gently positioned on its hind feet with its front paws on the elevated surface, and encouraged to jump by tossing treats, etc. If it still does not jump up, it can be gently lifted onto the surface, given treats, toys, petting, etc., then encouraged (as above) to jump down and to jump back up.*					
	Grade all criteria except 7 and 10 according to:	0	1	2	
1	*Body Posture – Back*				
	A. Lordosis (swayback)	None	Mild	Pronounced	
	B. Tendency to round/arch the back (convex)	No	Occasional	Frequent	
	C. Back has a dip just caudal to shoulders	No	Mild	Pronounced	
	D. Back has a thoracolumbar/lumbar/ lumbosacral hump	No	Mild	Pronounced	
	E. Front end lower than hind	No	Mild	Pronounced	
	F. Hind end lower than front	No	Mild	Pronounced	
Summed Section Score (add scores for 1A-1F)					
2	*Body Posture - Forelimbs*				
	A. Asymmetry (right to left)	No	Mild	Pronounced	
	B. Uneven weight distribution (right to left)	No	Mild	Pronounced	
	C. External rotation (toe out)	No	Mild	Pronounced	
	D. Limb abduction	No	Mild	Pronounced	
	E. Base wide appearance	No	Mild	Pronounced	
	F. Increased or decreased elbow flexion	No	Mild	Pronounced	
	G. Increased or decreased shoulder flexion	No	Mild	Pronounced	
Summed Section Score (add scores for 2A-G)					

MI-CAT(V)-v3 (continued)

3	*Body Posture – Hind limbs*				
	A. Asymmetry (right to left)	No	Mild	Pronounced	
	B. Uneven weight distribution (right to left)	No	Mild	Pronounced	
	C. External rotation (toe out)	No	Mild	Pronounced	
	D. Limb abduction	No	Mild	Pronounced	
	E. Base wide appearance	No	Mild	Pronounced	
	F. Hocks turned in/deviated medially	No	Mild	Pronounced	
	G. Hindquarters appear narrow from hips to hocks	No	Mild	Pronounced	
	H. Hind legs positioned forward under body	No	Mild	Pronounced	
	I. Increased or decreased hock flexion	No	Mild	Pronounced	
	J. Increased or decreased stifle flexion	No	Mild	Pronounced	
Summed Section Score (add scores for 3A-3J)					
4	*Gait - General*				
	A. Stiffness	No	Mild	Pronounced	
	B. Limp/decreased weight-bearing	No	Mild	Pronounced	
	C. Decreased speed of movement	No	Mild	Pronounced	
	D. Hindquarters deviate to one side (*e.g.*, at gallop)	No	Mild	Pronounced	
	E. Side to side movements of hips/pelvis/tail base	No	Mild	Pronounced	
	F. Lateral movements of spine at walk	No	Mild	Pronounced	
Summed Section Score (add scores for 4A-4F)					
5	*Gait - Forelimbs*				
	A. Stiffness	No	Mild	Pronounced	
	B. Shuffling gait	No	Mild	Pronounced	
	C. Limb circumduction	No	Mild	Pronounced	
	D. Heavy on front limbs	No	Mild	Pronounced	
	E. Shortened stride	No	Mild	Pronounced	
	F. Shoulder – reduced range of motion	No	Mild	Pronounced	
	G. Elbow – reduced range of motion	No	Mild	Pronounced	
Summed Section Score (add scores for 5A-5G)					
6	*Gait – Hind limbs*				
	A. Stiffness	No	Mild	Pronounced	
	B. Shuffling gait	No	Mild	Pronounced	
	C. Limb circumduction	No	Mild	Pronounced	
	D. Limbs appear to be carried far out behind the body	No	Mild	Pronounced	
	E. Shortened stride	No	Mild	Pronounced	
	F. Hip – reduced range of motion	No	Mild	Pronounced	
	G. Stifle – reduced range of motion	No	Mild	Pronounced	
	H. Hock – reduced range of motion	No	Mild	Pronounced	
	I. Does not track up with hind feet	No	Mild	Pronounced	
Summed Section Score (add scores for 6A-6I)					

MI-CAT(V)-v3 (continued)

7	*Willingness and Ease of Horizontal Movements*					
	A. Galloping	0 - Very willing	1 - Somewhat willing	2 - Reluctant	3 - Unwilling	
	B. Trotting	0 - Very willing	1 - Somewhat willing	2 - Reluctant	3 - Unwilling	
	C. Walking	0 - Very willing	1 - Somewhat willing	2 - Reluctant	3 - Unwilling	
	D. Preferred gaits (with encouragement)	0 - gallop/trot	1 – trot/walk	2 - walk		
	E. Decreased movement toward end of assessment	No	Mild	Pronounced		
	F. Switch direction	0 - Smooth pivot (hind limbs)	1 - Pivot, not smooth	2 - No pivot		
	G. Sits without encouragement	0 - No	1- Occasional	2- Frequent		
Summed Section Score (add scores for 7A-7G)						
8	*Standing up on Hind Feet to Investigate a Higher Surface*					
	A. Decreased ability	No	Mild	Pronounced		
	B. Intolerance of positioning by examiner (if needed)	No	Mild	Pronounced		
	C. Prefers this position to reach for treats (*vs*. jumping up)	No	Mild	Pronounced		
Summed Section Score (add scores for 8A-8C)						
9	*Jumping*					
	A. Requires encouragement to jump UP	No	Mild	Pronounced		
	B. Hesitates to jump UP	No	Mild	Pronounced		
	C. Effort when jumping UP	No	Mild	Pronounced		
	D. Use of front feet to aid jump UP	No	Mild	Pronounced		
	E. Awkward or clumsy jumping UP	No	Mild	Pronounced		
	F. Slinks down rather than jumping DOWN	Yes	Variable	Never		
	G. Jumps hind feet DOWN together	No	Variable	Usually		
	H. Flips hindquarters up in air when jumping DOWN	No	Mild	Pronounced		
	I. Lands heavily on hind feet when jumping DOWN	No	Mild	Pronounced		
Summed Section Score (add scores for 9A-9I)						
10	*Other Behaviors*					
	A. Tends to move along the edge of the room (wall)	0 - No	1 - Mild	2 - Pronounced		
	B. Grooms self during evaluation (in absence of known skin disease)	0 - No	1 - Yes			
	C. Stretches hind legs by lifting and extending backward one at a time	0 - No	1 - Yes			
Summed Section Score (add scores for 10A-10C)						
Total MI-CAT(V) Score (add summed section scores 1-10)						

5.3.2 Surgeon's Orthopedic Evaluation

SOE: Surgeon's Orthopedic Evaluation

1. Distance Evaluation:

Select the number from 0-10 corresponding to the severity of observed lameness.

Lameness Score	☐ 0 NO lameness	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10 WORST lameness imaginable

2. Hands-on Evaluation:

A. Vertebral Column

Note presence of reduced range of motion (ROM) where applicable (select Yes or No). Select the number from 0-10 corresponding to the severity of detected pain.

Spinal segment	Reduced ROM?		Pain
Cervical	Yes ☐	No ☐	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Thoracic	N/A	N/A	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Thoraco-lumbar	N/A	N/A	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Lumbar	N/A	N/A	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Lumbo-sacral/ Coccygeal	Yes ☐	No ☐	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable

SOE (continued)

B. Appendicular Joints

Note presence of palpable abnormalities other than pain (check box if abnormality is detected). Select the number from 0-10 corresponding to the severity of detected pain upon manipulation of the joint.

Joint	
Manus	Palpable Abnormality (*Check box if present*) ☐ Heat ☐ Edema ☐ Fibrosis ☐ Effusion ☐ Instability ☐ Crepitus ☐ ↓ROM Pain (*Indicate severity*) ☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Carpus	Palpable Abnormality (*Check box if present*) ☐ Heat ☐ Edema ☐ Fibrosis ☐ Effusion ☐ Instability ☐ Crepitus ☐ ↓ROM Pain (*Indicate severity*) ☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Elbow	Palpable Abnormality (*Check box if present*) ☐ Heat ☐ Edema ☐ Fibrosis ☐ Effusion ☐ Instability ☐ Crepitus ☐ ↓ROM Pain (*Indicate severity*) ☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Shoulder	Palpable Abnormality (*Check box if present*) ☐ Heat ☐ Edema ☐ Fibrosis ☐ Effusion ☐ Instability ☐ Crepitus ☐ ↓ROM Pain (*Indicate severity*) ☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable

SOE (continued)

Pes	Palpable Abnormality (*Check box if present*) ☐ Heat ☐ Edema ☐ Fibrosis ☐ Effusion ☐ Instability ☐ Crepitus ☐ ↓ROM Pain (*Indicate severity*) ☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Tarsus	Palpable Abnormality (*Check box if present*) ☐ Heat ☐ Edema ☐ Fibrosis ☐ Effusion ☐ Instability ☐ Crepitus ☐ ↓ROM Pain (*Indicate severity*) ☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Stifle	Palpable Abnormality (*Check box if present*) ☐ Heat ☐ Edema ☐ Fibrosis ☐ Effusion ☐ Instability ☐ Crepitus ☐ ↓ROM Pain (*Indicate severity*) ☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Hip	Palpable Abnormality (*Check box if present*) ☐ Heat ☐ Edema ☐ Fibrosis ☐ Effusion ☐ Instability ☐ Crepitus ☐ ↓ROM Pain (*Indicate severity*) ☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable

SOE (continued)

C. Long Bones

Note presence of physical abnormalities (select Yes or No). Select the number from 0-10 corresponding to the severity of detected pain upon palpation of the bone.

Long Bone	Physical Abnormality?		Pain (*Indicate severity*)
Metacarpals	Yes ☐	No ☐	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Radius/Ulna	Yes ☐	No ☐	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Humerus	Yes ☐	No ☐	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Metatarsals	Yes ☐	No ☐	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Tibia/Fibula	Yes ☐	No ☐	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable
Femur	Yes ☐	No ☐	☐ 0 NO pain ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST pain imaginable

3. Score Calculations:

Assign a value of 1 to each physical abnormality assessed as present. Assign NRS score selected for each pain score.

5.3.3 Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians – v4

MI-CAT(V)-v4 – Montreal Instrument for Cat Arthritis Testing (Veterinary) – Version 4

General Instructions

- *Perform the evaluation via distance observation, with the cat on the examination room floor.*
- *To stimulate walking, running, jumping, etc., toss treats or use toys (e.g., cotton ball, string) for it to chase, or call, gesture enticingly or pet the cat to encourage approach.*
- *DO NOT physically push or pull the cat, nor guide it by a leash or collar, nor startle it, as these will interfere with natural movements.*
- *Score criteria in order, or according to convenience based on the cat's unsolicited movements.*
- *The raised surface used for Criterion 6 should be ~15-24" tall (e.g. a bench, chair, shelf, or low table) so that the cat can investigate it without having to jump up.*
- *NOTE: Although each of the following criteria has the potential to be expressed in cats with osteoarthritis (OA), it is not expected that all will be present in a single animal.*

Evaluation Procedure

1) *Place the cat on the floor and observe its undisturbed behavior, posture, and movements. Assess posture (Criteria 1-3) both with the cat standing still and during locomotion.*
2) *If needed, encourage the cat to walk, run, turn, etc., so that gait can be observed from all perspectives (including from above) and so that willingness to move about can be assessed (Criteria 4-5). Also observe the gait as the cat passes under a low overhead obstacle (e.g., rungs of a chair or table), using encouragement if needed.*
3) *Encourage the cat to investigate a raised horizontal surface (Criterion 6). If the cat neither jumps up, nor places its front paws up, it can be gently positioned on its hind feet with its front paws on the raised surface, and encouraged to jump by tossing treats, etc. If the cat still does not jump up, it can be gently lifted onto the surface, given treats, petting, etc., then encouraged to jump down and to jump back up.*
4) *Note the presence/absence of the miscellaneous behaviors listed in Criterion 7.*
5) *If possible, lay the cat gently on its side on the floor or the table, and watch how it rises. Finally, complete the General Lameness Score (Criterion 8) based on your global subjective impression.*

Criteria Scoring

None (-)	*Mild (+)*	*Moderate (++)*	*Pronounced (+++)*
Unless otherwise indicated, scoring is as follows:	*(-) = 0,*	*(+) = 1,*	*(++) = 2,*	*(+++) = 3.*

**If any item cannot be scored (e.g. cat refuses to jump up/down for Criterion 6), the maximum possible score for the unscored item will be deducted from the total possible score in the final score calculation.*

#	Assessment Criteria	Response Options				Score
1	*Body Posture – Back*					
	A. Back has a dip just caudal to shoulders	-	+	++	+++	
	B. Back has a T-L/lumbar/L-S hump	-	+	++	+++	
	C. Front end lower than hind	-	+	++	+++	
	D. Hind end lower than front	-	+	++	+++	
Summed Section Score (add scores for 1A-D)						
2	*Body Posture – Forelimbs*					
	A. Asymmetry (right to left)	-	+	++	+++	
	B. Uneven weight distribution (right to left)	-	+	++	+++	
	C. Limb abduction	-	+	++	+++	
	D. External rotation (toe(s) turned out)	-	+	++	+++	
	E. Increased forelimb flexion	-	+	++	+++	
	F. Increased forelimb extension	-	+	++	+++	
Summed Section Score (add scores for 2A-F)						

MI-CAT(V)-v4 (continued)

3	*Body Posture – Hind limbs*					
	A. Asymmetry (right to left)	-	+	++	+++	
	B. Limb abduction	-	+	++	+++	
	C. Base wide appearance	-	+	++	+++	
	D. External rotation (hock(s) turned in)	-	+	++	+++	
	E. Stands with hind limbs held far forward under body	-	+	++	+++	
	F. Increased hind limb flexion	-	+	++	+++	
	G. Increased hind limb extension	-	+	++	+++	
Summed Section Score (add scores for 3A-G)						
4	*Gait*					
	A. Appears to move slowly	-	+	++	+++	
	B. Lateral movements of spine at walk	-	+	++	+++	
	C. Forelimb stiffness/lameness	-	+	++	+++	
	D. Forelimb circumduction	-	+	++	+++	
	E. Forelimb joints – reduced range of motion	-	+	++	+++	
	F. Hind limb stiffness/limping	-	+	++	+++	
	G. Hind limb circumduction	-	+	++	+++	
	H. Hind limb joints – reduced range of motion	-	+	++	+++	
Summed Section Score (add scores for 4A-H)						
5	*Willingness and Ease of Horizontal Movements*					
	*Note scoring change	0	1	2	3	
	A. How willing to walk?	Very	Somewhat	Reluctant	Unwilling	
	B. How willing to trot?	Very	Somewhat	Reluctant	Unwilling	
	C. How willing to gallop?	Very	Somewhat	Reluctant	Unwilling	
	D. Decreased locomotion towards end of assessment	-	+	++	+++	
	*Note scoring change	0	1	2		
	E. When encouraged to move about, cat usually:	Gallops/trots	Trots/walks	Walks		
	F. Reverses direction by pivoting quickly/smoothly on hind legs	Yes	Somewhat	No		
	G. Sits down (NOT a crouch) without encouragement	Never	Occasionally	Frequently		
	H. Goes into a sit (NOT a crouch) from standing:	Never	Lightly	Heavily		
Summed Section Score (add scores for 5A-H)						

MI-CAT(V)-v4 (continued)

6	*Jumping*						
	A. Prefers to stand on hind limbs to reach for treats	(No interest in treats*)	-	+	++	+++	
	B. Requires encouragement to jump UP	(Refuses*)	-	+	++	+++	
	C. Hesitates when jumping UP	(Refuses*)	-	+	++	+++	
	D. Uses front feet to aid jump UP	(Refuses*)	-	+	++	+++	
	E. Seems to hesitate/prepare to jump DOWN	(Refuses*)	-	+	++	+++	
	F. Hind feet land heavily (audibly/visibly) when jumping DOWN	(Refuses*)	-	+	++	+++	
	*Note scoring change	0		1		2	
	G. Slinks (with gliding steps) rather than jumping DOWN	Yes		Variable		Never	
	H. Hind feet land simultaneously when jumping DOWN	No		Variable		Usually	
Summed Section Score (add scores for 6A-H)							
7	*Other Behaviors*						
	A. Tends to move along the edge of the room (wall)	-	+	++	+++		
	B. Stretches hind legs by lifting and extending backward one at a time	-	+	++	+++		
Summed Section Score (add scores for 7A-B)							
8	*Global Distance Examination – General Lameness Score*						
	☐ 0 NO lameness ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST possible OA-related lameness						

Final Score Calculation

Add Section Scores 1-8 a =

* Add the maximum possible scores for all unscored/incomplete items b =

Subtract the maximum score for all unscored items (b) from the total possible scale score of 133: 133 - b:_____ c =

Sample Calculation:

Fluffy the cat has the following section scores: 1) 6, 2) 4, 3) 9, 4) 10, 5) 19, 6) 3, 7) 2, 8) 8. However, she would not jump up or down (items 6B-6H), and Dr. Smith forgot to score item 7A.

a = 6 + 4 + 9 + 10 + 19 + 3 + 2 + 8 = 61

b = 3 + 3 + 3 + 3 + 3 + 2 + 2 + 3 = 22

c = 133 – 22 = 111

Fluffy's Final Score = a ÷ c = 61 ÷ 111 = 55%

MI-CAT(V) Final Score = a ÷ c =

5.3.4 Montreal Instrument for Cat Arthritis Testing, for use by Veterinarians – v5

MI-CAT(V)-v5 – Montreal Instrument for Cat Arthritis Testing (Veterinary) – Version 5

General Instructions

- *Perform the evaluation via distance observation, with the cat on the examination room floor.*
- *To stimulate walking, running, jumping, etc., toss treats or use toys (e.g., cotton ball, string) for it to chase, or call, gesture enticingly or pet the cat to encourage approach.*
- *DO NOT physically push or pull the cat, nor guide it by a leash or collar, nor startle it, as these will interfere with natural movements.*
- *Score criteria in order, or according to convenience based on the cat's unsolicited movements.*
- *The raised surface used for Criterion 4 should be ~15-24" tall (e.g. a bench, chair, shelf, or low table) so that the cat can investigate it without having to jump up.*
- *NOTE: Although each of the following criteria has the potential to be expressed in cats with osteoarthritis (OA), it is not expected that all will be present in a single animal.*

Evaluation Procedure

1) *Place the cat on the floor and observe its undisturbed behavior, posture, and movements. Assess posture (Criterion 1) both with the cat standing still and during locomotion.*
2) *If needed, encourage the cat to walk, run, turn, etc., so that gait can be observed from all perspectives (including from above) and so that willingness to move about can be assessed (Criteria 2-3). Also observe the gait as the cat passes under a low overhead obstacle (e.g., rungs of a chair or table), using encouragement if needed.*
3) *Encourage the cat to investigate a raised horizontal surface (Criterion 4). If the cat neither jumps up, nor places its front paws up, it can be gently positioned on its hind feet with its front paws on the raised surface, and encouraged to jump by tossing treats, etc. If the cat still does not jump up, it can be gently lifted onto the surface, given treats, petting, etc., then encouraged to jump down and to jump back up.*
4) *If possible, lay the cat gently on its side on the floor or the table, and watch how it rises. Finally, complete the General Lameness Score (Criterion 5) based on your global subjective impression.*

Criteria Scoring

None (-)	*Mild (+)*	*Moderate (++)*	*Pronounced (+++)*
Unless otherwise indicated, scoring is as follows:	*(-) = 0,*	*(+) = 1,*	*(++) = 2,*	*(+++) = 3.*

**If any item cannot be scored (e.g. cat refuses to jump up/down for Criterion 4), the maximum score for the unscored item will be deducted from the maximum possible section score.*

#	Assessment Criteria	Response Options				Score
1	*Body Posture*					
	A. Back has a T-L/lumbar/L-S hump	-	+	++	+++	
	B. Stands with hind limbs held far forward under body	-	+	++	+++	
	C. Increased hind limb flexion	-	+	++	+++	
	*Note scoring change	0	1	2		
	D. Hind end lower than front	-	+	++/+++		
	E. Hind limb asymmetry (right to left)	-	+	++/+++		
	F. Forelimb asymmetry (right to left)	-	+	++/+++		
	G. Increased forelimb flexion	-	+	++/+++		
	*Note scoring change	0	1	2		
	H. Increased forelimb extension	Marked	Mild	None		
Summed Section Score (add cat scores for completed items 1A-H)						
Maximum Possible Section Score (19 – maximum scores for incomplete items)						

MI-CAT(V)-v5 (continued)

2	*Gait*						
	A. Appears to move slowly	-	+	++	+++		
	B. Forelimb stiffness/lameness	-	+	++	+++		
	C. Hind limb stiffness/lameness	-	+	++	+++		
	*Note scoring change	0	1	2			
	D. Forelimb joints – reduced range of motion	-	+	++/+++			
	E. Hind limb joints – reduced range of motion	-	+	++/+++			
	*Note scoring change	0	1	2			
	F. Lateral movements of spine at walk	-/+	++	+++			
	G. Hind limb circumduction	-/+	++	+++			
	Summed Section Score (add scores for 2A-G)						
	Maximum Possible Section Score (17 – maximum scores for incomplete items)						
3	*Willingness and Ease of Horizontal Movements*						
	*Note scoring change	0	1	2	3		
	A. How willing to trot?	Very	Somewhat	Reluctant	Unwilling		
	*Note scoring change	0	1	2			
	B. How willing to walk?	Very	Somewhat	Reluctant			
	C. Goes into a sit (NOT a crouch) from standing:	Heavily	Lightly	Never			
	*Note scoring change	0	1				
	D. Reverses direction by pivoting quickly/smoothly on hind legs	Yes	No				
	Summed Section Score (add scores for 3A-D)						
	Maximum Possible Section Score (8 – maximum scores for incomplete items)						
4	*Jumping*						
	*Note scoring change	(Unscored)	0	1	2		
	A. Requires encouragement to jump UP	(Refuses*)	-	+	++/+++		
	B. Hesitates when jumping UP	(Refuses*)	-	+	++/+++		
	C. Uses front feet to aid jump UP	(Refuses*)	-	+	++/+++		
	D. Seems to hesitate/prepare to jump DOWN	(Refuses*)	-	+	++/+++		
	*Note scoring change	(Unscored)	0	1	2		
	E. Hind feet land heavily (audibly/visibly) when jumping DOWN	(Refuses*)	-/+	++	+++		
	Summed Section Score (add scores for 4A-E)						
	Maximum Possible Section Score (10 – maximum scores for incomplete items)						

MI-CAT(V)-v5 (continued)

5	*Global Distance Examination – General Lameness Score*
	☐ 0 NO lameness ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 WORST possible OA-related lameness
Maximum Possible Section Score (10 if Section 5 is completed, 0 if incomplete)	

Final Score Calculation

Add Section Scores 1-5 a =

* Add the maximum possible scores for Sections 1-5 (maximum of 64) b =

Sample Calculation:

Fluffy the cat would not jump up or down (items 4A-4E), and Dr. Smith forgot to score item 1F. She has the following section scores: 1) 12, 2) 10, 3) 8, 4) (no score), and 5) 8.

a = 12 + 10 + 8 + (0) + 8 = 38

b = (19-2) + 17 + 8 + (10-10) + 10 = 17 + 17 + 8 + 0 + 10 = 52

Fluffy's Final Score = *a* ÷ b = 38 ÷ 52 = 0.73

MI-CAT(V) Final Score = a ÷ b =